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Developmental Expression of Steroidogenic Factor 1 in a Turtle with Temperature-Dependent Sex Determination

Alice Fleming,* Thane Wibbels,† James K. Skipper,* and David Crews*,[1]

*Section of Integrative Biology, University of Texas at Austin, Austin, Texas 78712; and †Department of Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294-1170

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A variety of reptiles possess temperature-dependent sex determination (TSD) in which the incubation temperature of a developing egg determines the gonadal sex. Current evidence suggests that temperature signals may be transduced into steroid hormone signals with estrogens directing ovarian differentiation. Steroidogenic factor 1 (SF-1) is one component of interest because it regulates the expression of steroidogenic enzymes in mammals and is differentially expressed during development of testis and ovary. Northern blot analysis of SF-1 in developing tissues of the red-eared slider turtle (*Trachemys scripta*), a TSD species, detected a single primary SF-1 transcript of approximately 5.8 kb across all stages of development examined. Analysis by *in situ* hybridization indicated nearly equivalent SF-1 expression in early, bipotential gonads at male (26°C)- and female (31°C)-producing incubation temperatures. In subsequent stages, as gonadal sex first becomes histologically distinguishable during the temperature-sensitive period, SF-1 expression increased in gonads at a male-producing temperature and decreased at a female-producing temperature, suggesting a role for SF-1 in the sex differentiation pathway. SF-1 message was also found in adrenal and in the periventricular region of the preoptic area and diencephalon, but there was no apparent sex bias in these tissues at any stage examined. The overall developmental pattern of SF-1 mRNA expression in *T. scripta* appears to parallel that found in mammals, indicating possible homologous functions.

Key Words: steroidogenic factor 1, SF-1; Ad4BP; FTZ-F1; reptile; turtle; *Trachemys scripta;* temperature-dependent sex determination.

INTRODUCTION

Gonadal sex of species with temperature-dependent sex determination (TSD) is determined by the temperature at which their eggs are incubated. In the red-eared slider turtle (*Trachemys scripta*), only males are produced when eggs are incubated at 26°C, and only females are produced at 31°C (Bull *et al.*, 1982). The temperature-sensitive period (TSP) for sex determination occurs between Yntema (1968) stages 15 and 21, the middle third of incubation (Wibbels *et al.*, 1991). Commitment to gonadal sex occurs within that period.

Sex steroid hormones are implicated in the process of TSD, and estrogen, in particular, appears essential in female sex determination (Crews, 1996; Crews *et al.*, 1994; Wibbels *et al.*, 1998; Lance, 1997). Estrogens applied exogenously to *T. scripta* eggs incubating at a male-producing temperature override the temperature effect, and female hatchlings result (Crews *et al.*, 1991; Wibbels and Crews, 1992). Exogenously applied inhibitors of aromatase—the enzyme that converts testosterone to estrogen (Simpson *et al.*, 1994)—override a female-producing incubation temperature, and male

[1] To whom correspondence should be addressed. Fax: (512) 471-6078. E-mail: crews@mail.utexas.edu.

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hatchlings result (Crews and Bergeron, 1994; Wibbels and Crews, 1994).

Work with other turtle species has shown a correlation between female incubation temperatures and increased levels of endogenous aromatase transcript and activity in the putative ovary during the TSP (Desvages and Pieau, 1992; Jeyasuria and Place, 1997, 1998). Other researchers have found aromatase activity in the turtle brain prior to that found in the gonad at female-producing temperatures and have proposed that the brain, rather than the gonad, is the sex-determining source of estrogen (Merchant-Larios, 1998; Jeyasuria and Place, 1998). Whatever the endogenous source of estrogen, gonads of putative females and males are receptive to its effect as both express estrogen receptor, albeit differentially, throughout the TSP (Bergeron *et al.*, 1998).

Male sex determination can be manipulated by exogenously applied dihydrotestosterone, a nonaromatizable androgen, and by inhibitors of its endogenous synthesis (Crews and Bergeron, 1994; Wibbels and Crews, 1992, 1995; Wibbels *et al.*, 1992). This effect is less striking than that of estrogen in female sex determination and is only seen at intermediate, or less potent, incubation temperatures. Nevertheless, steroid hormones are undoubtedly a part of TSD in both males and females.

To begin exploring the underlying molecular mechanisms of steroid hormones in TSD, we examined the distribution of steroidogenic factor 1 (SF-1) (Lala *et al.*, 1992), also called Ad4BP (Morohashi *et al.*, 1992), in *T. scripta*. SF-1, encoded by the FTZ-F1 gene and a member of the nuclear receptor superfamily, is known in mammals to regulate transcription of many genes within the reproductive axis (reviewed in Morohashi and Omura, 1996; Parker and Schimmer, 1997). In steroidogenic tissue, it regulates the gene activity of many proteins involved in the synthesis of testosterone and estrogen, including steroidogenic acute regulatory protein, $P450_{scc}$, $P450_{17\alpha}$, 3β-HSD, and aromatase (reviewed in Morohashi, 1999; Parker *et al.*, 1999). During mammalian development, SF-1 is differentially expressed in testes and ovaries (Ikeda *et al.*, 1994; Hatano *et al.*, 1994).

In this study we examined the pattern of SF-1 mRNA expression in *T. scripta*. Northern blot analysis was performed to determine presence and size of message and possible alternate transcripts. A single transcript was found in all stages and tissues examined. Adrenal, kidney, and gonad cannot be effectively separated at early developmental stages in *T. scripta*, precluding traditional quantitative measures of SF-1 in gonad alone. For this reason, *in situ* hybridization was selected as the most appropriate technique to both localize and quantify SF-1 in the embryonic turtle. SF-1 message was found in similar amounts and distribution in the bipotential gonad of males and females. During stages when the sex of gonads is becoming distinct and committed, SF-1 message increased at a male-producing temperature and decreased at a female-producing temperature. SF-1 message was also detected in developing adrenal and the periventricular region of the preoptic area and diencephalon in similar amounts and distribution at male- and female-producing temperatures. The sex-based differential expression of SF-1 in the turtle gonad during a critical period of gonadal sex development mirrors that found in mammals, suggesting homologous functions and possible involvement in temperature-sensitive sex determination and differentiation.

MATERIALS AND METHODS

Tissue Collection

T. scripta eggs were purchased within 2 days of laying from Robert Kliebert (Kliebert Turtle Farms, Hammond, LA), brought to the laboratory, and kept at room temperature until viability was established by candling. Viable eggs were placed in containers with moistened vermiculite (1:1 vermiculite to water) and randomized across containers to eliminate clutch effects. The containers were placed in incubators (Precision, Chicago) at either 26 or 31°C. Continuous incubation of *T. scripta* eggs at 26°C produces all male hatchlings whereas incubation at 31°C produces all female hatchlings (Bull *et al.*, 1982).

Temperature of the incubators was continuously monitored with HOBO data loggers (Onset Computer Corp.), supplemented by daily checks of in-incubator thermometers. Temperature fluctuations were less than 0.1°C. Egg boxes were rotated within the incubators each day, and eggs were checked periodically for

developmental stage according to Yntema's staging guidelines (1968). By these guidelines, the temperature-sensitive period in *T. scripta* is from approximately stage 15 through 21, and eggs hatch at stage 26.

For *in situ* hybridization, embryos were taken at stages 13 through 19 and at stage 23 from each incubation temperature, quickly frozen on dry ice, and stored at −80°C until sectioning. For all other molecular work, embryos were decapitated; the adrenal–kidney–gonad (AKG) complex and brain were then quickly dissected out, frozen in liquid nitrogen or isopentane, and stored at −80°C until use.

Probe Preparation

The open reading frame of *T. scripta* SF-1 cDNA has been cloned (Cowan, J., 1998, M. S. thesis, University of Alabama at Birmingham; GenBank Accession No. AF033833; Wibbels *et al.*, 1998). Cowan and Wibbels provided us a 457-bp clone spanning exon 5 (8 bp only) and exons 6a, 7, and 8 (185 bp only) (gene structure according to Ninomiya *et al.*, 1995). At its 5′ end, this clone includes 125 bp common to SF-1 and ELP1, an alternate transcript of FTZ-F1 (Ikeda *et al.*, 1993), and was, therefore, used in its entirety as the riboprobe template for Northern blot analysis. β-actin was cloned by reverse transcriptase polymerase chain reaction (RT-PCR) using RNA isolated per RNAgents Total RNA Isolation System (Promega) from stage 23 *T. scripta* tissue incubated at a male-producing temperature (26°C). Degenerate primers were provided by K. Gen through Peter Thomas (University of Texas Marine Science Institute, Port Aransas, TX). SF-1/ELP1 and β-actin vectors were linearized, and riboprobes were made by run-off transcription using the RNA Strip-EZ System from Ambion and 32P-UTP from NEN. Probes were synthesized to a specific activity of 8×10^7 cpm/μg and used at a final concentration of 5×10^8 cpm/ml of hybridization solution.

Riboprobe templates for *in situ* hybridization were prepared by subcloning the Cowan and Wibbels SF-1/ELP1 clone, in both sense and antisense orientations, into pCRII vector (Invitrogen) to eliminate sequence in common with ELP1. These 330-bp subclones contain all of exon 7 and the 5′ end of exon 8 (Ninomiya *et al.*, 1995). Riboprobes were made by run-off transcription with enzymes from New England BioLabs and 35S-CTP from NEN, using protocols of Sambrook *et al.* (1989). Probes were synthesized to a specific activity of 9×10^8 cpm/μg and were used at a concentration of 0.3 μg probe × length (kb)/ml hybridization solution.

Northern Blot Analysis

Total RNA was isolated according to Sambrook *et al.* (1989) or RNAgents Total RNA Isolation System. *T. scripta* tissues were AKG complexes from early, middle, and post TSP (stages 15, 18/19, and 23, respectively) at both male- and female-producing temperatures (26 and 31°C); whole brain from the middle of the TSP (stages 18/19) at both temperatures; and adult ovary. Twenty-five micrograms of total RNA from each tissue and RNA Millenium Markers (Ambion) were loaded. The blot was prepared using Ambion's Northern Max kit and BrightStar-Plus membrane. After hybridization with the SF-1 probe the blot was stripped and rehybridized with the β-actin probe using RNA Strip-EZ. Bound probe was visualized by phosphorimager (Molecular Dynamics) using ImageQuant software.

In Situ Hybridization

Three individuals per temperature/stage were analyzed. Frozen, whole torsos or AKGs (stages 14, 16–18, and 23) or heads (stages 13 through 19 plus 23) were embedded in OCT compound (Tissue Tek) and sectioned on a cryostat (2800 Frigocut, Reichert-Jung) at 20 μm. Sections were placed serially on sets of four poly-L-lysine-treated slides, air dried, and stored at −80°C. The *in situ* hybridization protocol used in our laboratory has been previously described (Young *et al.*, 1994). After hybridization with SF-1 antisense or sense (for negative control) probe, slides were dipped in Kodak NTB-2 autoradiographic emulsion and exposed at 4°C for 10 days. They were then developed (Kodak D19 Developer), fixed (Kodak Fixer), and stained with Harris hematoxylin for tissue in the torso or cresyl violet for tissue in the head. Darkfield quantification of silver grains in specifically labeled cells, defined as having a density of silver grains at least three times that of background, was done as previously described in Bergeron *et al.* (1998). Briefly, slides were computer coded and randomized to prevent bias during measurement. Measurement was done using the Grains Counting Program (University of Washington). The 45 most densely labeled clusters (each approximating the size

of a single cell) of gonad per individual were automatically selected and the number of silver grains per cluster counted by the Grains program. To measure tissue-based background labeling, the system was then asked to select and count grains per cluster in the adjacent kidney of each individual. The average background count per cluster was subtracted from the average gonad count per cluster. Corrected measures for the three individuals in each stage/temperature were then averaged and used in the findings below. Further statistical analysis was not done due to the small sampling size.

RESULTS

Northern Blot Analysis

A single primary band of approximately 5.8 kb was found in each of the *T. scripta* tissues examined: stages 15, 18/19, and 23 in the adrenal–kidney–gonad complex (Fig. 1) and stage 18/19 in the brain (data not shown), at both incubation temperatures. The β-actin

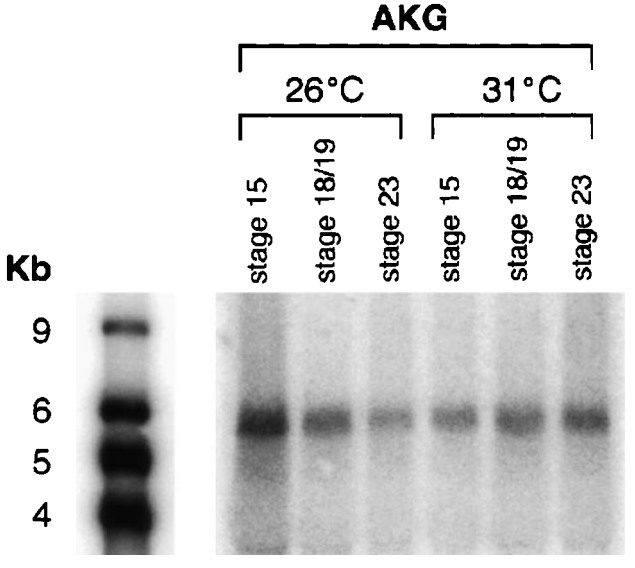

FIG. 1. Northern blot analysis of SF-1 mRNA developmental expression in adrenal–kidney–gonad tissue of *T. scripta*. Lane 1: marker. Lanes 2–7: approximate beginning of the TSP (stage 15), during the TSP (stages 18/19), and after the TSP (stage 23) tissue from male (26°C)- and female (31°C)-producing incubation temperatures. Conditions of the experiment did not allow quantification.

results indicated that quality of the RNA was preserved and loading and transfer were approximately even (data not shown). SF-1 is strongly expressed in adrenal as well as in gonad (*in situ* hybridization observation), and band intensities reflect expression in combined tissue types. Therefore, quantification of SF-1 expression in individual tissues could not be done.

In Situ Hybridization

In the torso, SF-1 mRNA was detected in the adrenal and gonad of all stages assayed (stages 14, 16–18, and 23) and at both 26 and 31°C. No other tissues in the torso showed signal above background. Signal in the adrenal, although more intense than in the gonad, appeared the same at male- and female-producing temperatures, indicating no sex bias in that tissue (data not shown).

In the gonad, SF-1 signal was clearly visible at both incubation temperatures in the earliest stage examined, stage 14, which occurs prior to the temperature-sensitive period. Expression levels were nearly equivalent at male- and female-producing temperatures (Fig. 2). At this stage, gonads from both temperatures are bipotential and gonadal sex is histologically indistinguishable; gonad and adrenal tissue are distinct.

At approximately stages 18/19, gonadal sex can first be detected histologically in *T. scripta* embryos. Between stages 17 and 18, SF-1 expression rose in putative testes but decreased slightly in putative ovaries (Fig. 2).

At stage 23, a stage well after the TSP, the largest difference in gonadal expression was seen. SF-1 message dropped close to background in gonads at the female-producing temperature but remained high at the male-producing temperature.

A difference in the distribution of SF-1 in *T. scripta* gonad also emerged between sexes over time (Fig. 3). At stage 14, SF-1 mRNA was evenly dispersed throughout the gonad at both incubation temperatures (Figs. 3a and 3d). During stages 17 and 18, signal appeared clustered into striations at the male-producing temperature (Fig. 3b), coincident with early organization of medullary cords. SF-1 signal in most putative ovaries was evenly dispersed at stages 17 and 18 (Fig. 3e), though signal in two individuals was organized in a faint cord-like pattern. In *T. scripta*, medullary cords begin forming in putative females as well as males during this time (Wibbels *et al.*, 1991).

During stages 18 through 20, medullary cords prolif-

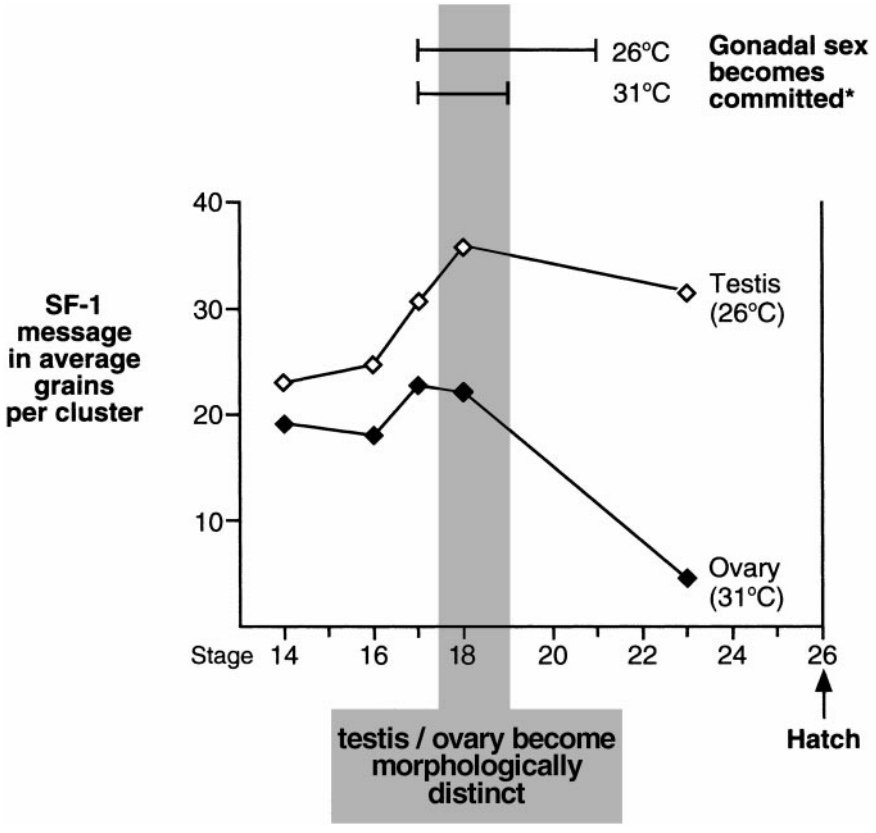

FIG. 2. SF-1 message expressed in developing gonad of *T. scripta* at male (26°C)- and female (31°C)-producing temperatures, determined by *in situ* hybridization. Each data point represents a simple mean of three individuals (corrected for background). Ranges, standard errors, and statistical comparisons are omitted because of small sample size. *For comparative purposes, we have included approximate timelines for commitment to gonadal sex at male (26°C)- and female (31°C)-producing temperatures, based on Wibbels *et al.* (1991).

erate at male-producing temperatures and regress at female-producing temperatures (Wibbels *et al.*, 1991). By stage 23 in putative testes, medullary distribution of SF-1 signal was clearly organized in or around medullary cords (Figs. 3c and 4). In both compartments of the testes—medullary cords and interstitial space—SF-1 signal was above background (Fig. 4b). Signal in one of the compartments was clearly stronger than in the other but markers to distinguish compartments were not used in this experiment. In sharp contrast, SF-1 signal in putative ovaries at stage 23 was close to background and found only in the cortical region (Fig. 3f). Signal in the medullary region, which is largely vacuolated by this stage, was below tissue-based background as measured in the kidney.

In sections of embryonic *T. scripta* head, SF-1 mRNA was present (Fig. 5) in all developmental stages assayed (stages 13 through 19 plus 23) at both incubation temperatures. Message was localized in the periventricular region of the preoptic area and diencephalon (reference atlases were Harless and Morlock, 1979; Young *et al.*, 1994; Powers and Reiner, 1980; Kandel *et al.*, 1991). Signal in this region extended over many tissue sections, rostral to caudal, in each individual. There was no apparent sex-based difference in amount or distribution of SF-1 message. No signal above background was seen in torso or head tissues probed with labeled sense strand.

DISCUSSION

Steroidogenic factor 1 has now been identified in several mammals (Lala *et al.*, 1992; Morohashi *et al.*, 1992; Lynch *et al.*, 1993; Wong *et al.*, 1996; Pilon *et al.*,

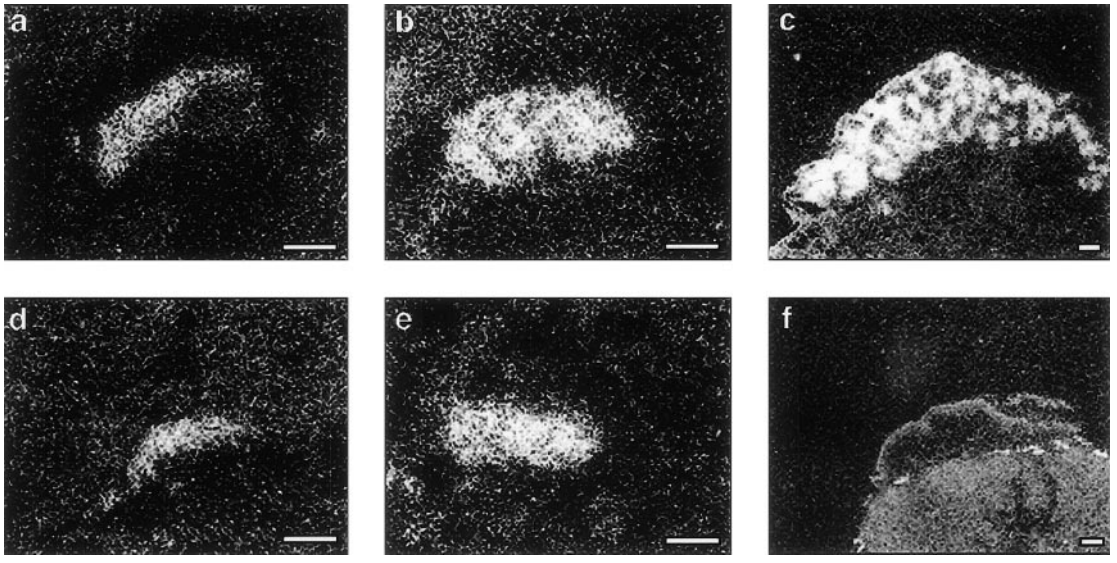

FIG. 3. *In situ* hybridization of SF-1 probe to embryonic gonadal tissue of *T. scripta* (cross section). (a, b, c) Male-producing incubation temperature (26°C). (d, e, f) Female-producing temperature (31°C). (a, d) Before the TSP (stage 14). (b, e) During the TSP (stage 18). (c, f) After the TSP (stage 23). Bar, 100 μm.

1998), the chicken (Kudo and Sutou, 1997; Smith *et al.*, 1999), and two TSD reptiles—the American alligator (P. Western and A. Sinclair, personal communication) and *T. scripta* (Wibbels *et al.*, 1998). The pattern of SF-1 expression in early *T. scripta* gonadal development resembles that of all other amniotes examined to date: SF-1 message is present from the earliest urogenital ridge throughout the bipotential (or indifferent) phase with no apparent sex bias (Ikeda *et al.*, 1994; Hatano *et al.*, 1994; Smith *et al.*, 1999; P. Western, personal communication). This implies conserved function. In mammals and chickens, expression of SF-1 in the gonad significantly precedes expression of known SF-1 target steroidogenic genes (Parker and Shimmer, 1997; Smith *et al.*, 1999), which suggests its involvement in nonsteroidogenic functions during early devel-

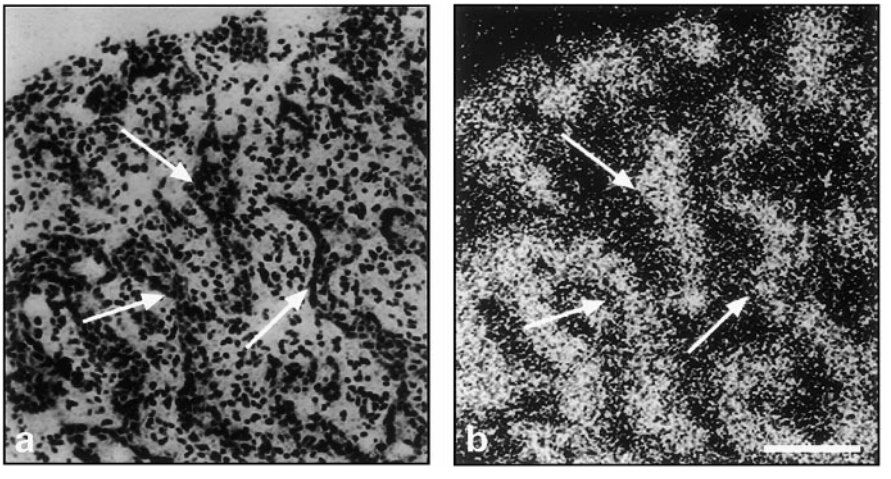

FIG. 4. Lightfield (a) and darkfield (b) *in situ* hybridization images of SF-1 mRNA expression in the two compartments of post-TSP (stage 23). *T. scripta* testis. Arrows indicate comparable points on a and b. Background signal is visible in upper left corner. Bar, 100 μm.

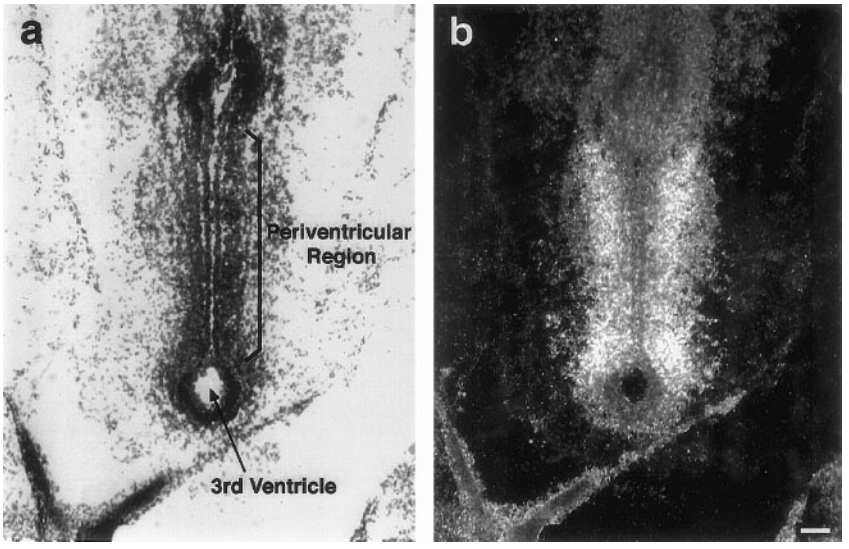

FIG. 5. *In situ* hybridization analysis of the periventricular region of the diencephalon and preoptic area of *T. scripta* brain (coronal section). Tissue is from a male-producing incubation temperature (26°C) during the TSP (stage 18). (a) lightfield and (b) darkfield images. Bar, 100 μm.

opment of the gonad. Two separate lines of research indicate that SF-1 may be involved in the primary differentiation and/or maintenance of steroidogenic tissues. Stable expression of SF-1 in murine embryonic stem cells induces cell differentiation to the point of synthesizing progesterone (Crawford *et al.*, 1997). Ftz-F1-disrupted neonatal mice show complete agenesis of gonad and adrenal with indications of apoptosis (Luo *et al.*, 1994). In *T. scripta*, the period of bipotential gonad development extends into the approximate beginning of the TSP (stages 15/16), during which stages there is a temperature effect, but commitment to gonadal sex has not yet occurred (Wibbels *et al.*, 1991).

As gonadal sex first becomes histologically distinct, the pattern of SF-1 expression among amniotes appears to diverge. In *T. scripta*, gonadal sex can first be distinguished at developmental stages 18/19. Between stages 17 and 18, SF-1 message increases at the male-producing temperature while tapering off at the female-producing temperature. As found in a separate *in situ* hybridization, this pattern continued at stage 19 (data not shown). At the male-producing temperature of 26°C, commitment to gonadal sex starts at approximately stage 17 (3% of individuals are committed) and is fixed for 100% of individuals by stage 21. At the female-producing temperature of 31°C, the period of commitment also begins at stage 17 (20% are fixed), but 100% of females are committed by stage 19 (Wibbels *et al.*, 1991). These stages can vary slightly with the exact incubation regimen and due to clutch effect. Nevertheless, differential expression of SF-1 appears to begin at about the time both morphological distinction and commitment to gonadal sex are occurring (Fig. 2). At stage 23, after the TSP, SF-1 expression is markedly higher in males than in females (Figs. 2, 3c, and 3f).

A similar pattern is found in mammals in that SF-1 expression becomes differential in developing gonads just as testes and ovaries become distinguishable (Hatano *et al.*, 1994; Ikeda *et al.*, 1993). At embryonic day 12.5 (E12.5) in mice, expression is high in males and very low in females. This difference continues, coincident with rapid testicular differentiation, until late in gonadal development (Ikeda *et al.*, 1994).

SF-1 expression in *T. scripta* and mammals is evident in both compartments of developing testes. *In situ* hybridization darkfield images of mouse and rat indicate a higher level of SF-1 in the interstitial space than in testicular cords (Hatano *et al.*, 1994; Ikeda *et al.*, 1994) and appear quite similar to those of *T. scripta* (Fig. 4). In mammals, SF-1 is thought to regulate transcription of Müllerian inhibiting substance (MIS) in Sertoli cells in the testicular cords (Shen *et al.*, 1994; Giuili *et al.*, 1997) and synthesis of testosterone in Leydig cells in the interstitial space (Ikeda *et al.*, 1993; Hatano *et al.*, 1994). MIS has been cloned in *T. scripta* and, though its overall developmental expression pattern is not yet known, it

is present in putative testes at stage 23 (Wibbels *et al.*, 1998), a time roughly comparable to its expression in mammals. SF-1 expression in putative testes of *T. scripta* is also high at this stage. The conserved pattern of SF-1 expression in *T. scripta* and mammals suggests homologous functions in male gonadal sex development.

In chicken and alligator, SF-1 expression following histological distinction of gonadal sex differs from that in *T. scripta* and mammals. SF-1 levels become less abundant in testes than ovary in the genetically sex-determined chicken (Smith *et al.*, 1999) and temperature sex-determined alligator (P. Western, personal communication).

In chicken, SF-1 message expression falls to an almost negligible level in males while remaining high in females. Smith *et al.* (1999) found that increased SF-1 expression in the chicken ovary correlates with its high level of aromatase expression (Andrews *et al.*, 1997; Smith *et al.*, 1997). Aromatase is regulated by SF-1 in mammalian granulosa cells (Carlone and Richards, 1997), where it converts testosterone to estrogen. Estrogen is essential to development of chicken ovary, where it is synthesized at high levels (Imataka *et al.*, 1988; Woods and Erton, 1978). In eutherian mammals, on the other hand, estrogen is apparently not essential to ovarian development (Lubahn *et al.*, 1993; Greco and Payne, 1994), aromatase is rarely detected in the developing ovary (Greco and Payne, 1994), and SF-1 levels are very low at this time (Hatano *et al.*, 1994; Ikeda *et al.*, 1994).

In alligator, the level of SF-1 mRNA is consistently lower in putative males than females from the middle of the TSP onward, as detected by RT-PCR (P. Western, personal communication). Many lines of research implicate estrogen in female sex determination of TSD reptiles (reviewed in Crews, 1996; Lance, 1997; Wibbels *et al.*, 1998), including alligators. The relative abundance of SF-1 in putative female alligators compared to putative males could regulate increased aromatase expression and subsequent estrogen synthesis. However, aromatase activity has not been detected in developing female alligator adrenal–kidney–gonad complex until after the TSP and gonadal determination (Smith *et al.*, 1995).

Estrogen is considered essential to female sex determination in *T. scripta* as well (Crews and Bergeron, 1994). However, SF-1 expression in developing ovaries of *T. scripta* appears to decline, unlike that of chicken and alligator, as histological sex becomes distinct (stages 18/19). This is a critical point in *T. scripta* female sex determination, in that gonadal sex appears committed in 100% of individuals at this time (Wibbels *et al.*, 1991). It may be that estrogen is required for only a short time as part of a female cascade, in which case SF-1 would not be needed for ongoing expression of aromatase. Indeed, a single dose of estrogen applied exogenously at stage 17 to *T. scripta* eggs incubating at an all-male-producing temperature results in all female hatchlings (Crews *et al.*, 1991). Here, we find levels of SF-1 in putative ovary do not begin to fall until after stage 17.

Interestingly, Majdic *et al.* (1997) report that subcutaneous injections of estrogen in pregnant rats at E11.5 and E15.5 result in significant reduction of gonadal SF-1 message in genotypic male embryos recovered at E17.5. Were a related mechanism present in *T. scripta*, exogenous application of estrogen or endogenous production in ovary or other tissues could feed back negatively on SF-1 expression in the ovary.

SF-1 is strongly expressed in the adrenal of *T. scripta*, raising the question of estrogen synthesis in that tissue rather than, or in addition to, gonad during female sex determination. We found no apparent sex bias in SF-1 expression in the adrenal and, therefore, no direct support for differential aromatase expression in that tissue.

There is recent evidence suggesting the brain rather than, or in addition to, gonad or adrenal may be involved in sex determination in TSD turtles. Merchant-Larios (1998) found estrogen levels in midbrain of sea turtle significantly higher at female- than at male-producing temperatures during the thermosensitive period. Jeyasuria and Place (1998) found aromatase transcript in brain of putative male and female diamondback terrapin before it was detectable in gonad, and more abundant in putative females than males early in the TSP. Here, we report SF-1 expression in brain before, during, and after the TSP with no apparent sex bias. If aromatase is differentially expressed in *T. scripta* brain at male- and female-producing temperatures during the TSP, its regulation must involve differential posttranscriptional regulation of SF-1 or other regulatory factor(s) altogether.

SF-1 in developing *T. scripta* brain may be involved in other functions. In Ftz-F1-disrupted mice, the structure of the ventromedial hypothalamus is malformed (Ikeda *et al.,* 1995; Shinoda *et al.,* 1995), implying a role for SF-1 in neural development. We detected SF-1 in this region of *T. scripta* brain. In the anterior pituitary of wild-type adult mice, SF-1 has been implicated in transcriptional regulation of gonadotropins and the GnRH receptor (reviewed in Parker and Schimmer, 1997). It is tempting to suggest fetal SF-1 regulation of estrogen by way of these proteins in *T. scripta.* However, no SF-1 expression was detected in the developing pituitary.

Is SF-1 involved in sex determination and/or differentiation in *T. scripta?* SF-1 is expressed at male- and female-producing temperatures prior to and early in the TSP but it is differentially expressed only in gonad and only as gonadal sex becomes distinct. This suggests that SF-1 is not, by itself, a sex-determining gene. There is, however, enough evidence compiled to suggest that it may be one critical component in gonadal sex development, perhaps regulating MIS expression and testosterone synthesis at male-producing temperatures and aromatase expression at female-producing temperatures.

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