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TEMPERATURE VARIATION AND SEX DETERMINATION IN REPTILIA

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Abbreviations:

A, androstenedione
Arom, aromatase
AMH, anti - Müllerian hormone
cAMP, adenosine 3': 5'- cyclic monophosphate
DHT, dihydrotestosterone
E, estrogen
E1, estrone
E2, estradiol - 17B
ER, estrogen receptor
FT, female-producing temperature
3B-HSD, 3B-hydroxysteroid dehydrogenase-5-ene-4-ene isomerase
17B-HSD, 17B-hydroxysteroid dehydrogenase
hsp, heat-shock protein
MT, male-producing temperature
P 450 arom, aromatase cytochrome P 450
P 450 scc, cholesterol side-chain-cleavage cytochrome P 450
5alpha-red, 5alpha-reductase
T, testosterone

Summary

In many species of reptiles, sex is determined by sex chromosomes as early as union of gametes (male or female heterogamety). In other species, including all crocodilians, most turtles and some lizards studied so far, sex is influenced by temperature during the earlier stages of gonadal differentiation. The effects of exogenous estrogens, antiestrogens and aromatase inhibitors at different temperatures have irrefutably demonstrated the involvement of estrogens in sexual differentiation of the gonads. Aromatase is the enzyme converting androgens to estrogens. Gonadal aromatase activity is well correlated with gonadal structure. It increases exponentially in differentiating ovaries, whereas it remains low in differentiating testes. Moreover, there is a high concordance between the thermosensitive periods for both ovary differentiation and increase in aromatase activity. It is suggested that a thermosensitive factor intervenes, directly or indirectly, in the transcriptional regulation of the aromatase gene in reptiles with temperature-dependent sex determination.

Introduction

In many species of reptiles as in most vertebrates, sex is fixed as early as fertilization by (a) gene(s) carried out by (a) sex chromosome(s) and sex ratio at hatching is generally 1 male : 1 female. The so-called "Genotypic sex-determination" (GSD)[1,2] or "Chromosomal sex-determination" (CSD)[3] obeys two main systems, male heterogamety (male XY, female XX) as in mammals and female heterogamety (female ZW, male ZZ) as in birds[4]. These systems are not necessarily accompanied by heteromorphism of sex chromosomes[5,6]. Thus, in snakes which exhibit a ZW/ZZ system, sex chromosomes present various degrees of differentiation from homomorphism (in boas, pythons, blind snakes) to heteromorphism (in colubrids, viperids and elapids). All cases of heteromorphism in snakes seem to be

homologous, involving the same (fourth) pair of chromosomes[2,5]. Both XX/XY and ZW/ZZ systems and multiple sex chromosomes systems, with or without heteromorphism of sex chromosomes, exist in lizards[2,6]. In turtles, male heterogamety or female heterogamety, generally with a slight heteromorphism of sex chromosomes, has been observed in a few species[2,6,7].

Not all reptilian species are sex fixed at fertilization. In a number of them, increasing with new investigations, the sexual differentiation of gonads has been shown to be sensitive to the incubation temperature of eggs during a critical period of embryonic development. This phenomenon has been called "Temperature-dependent sex determination" (TSD)[1,2].

That temperature would influence the sex ratio of the hatchlings in reptiles was suggested for the first time in 1966 from observations in a lizard from West Africa, *Agama agama*[8]. In 1971-72, TSD was discovered in the European freshwater turtle (*Emys orbicularis*) and a Mediterranean tortoise (*Testudo graeca*)[9,10]. A few years later, a paper documenting TSD in a turtle (*Chelydra serpentina*) living in the USA was published[11]. This paper seems to have stimulated the interest in this topic. Indeed, from the end of the seventies, number of publications from different laboratories showed that TSD is widespread in reptiles, and that it occurs in natural as in artificial conditions. Among species studied so far, this phenomenon has been found in all crocodilians, most turtles and some lizards, but not in snakes (reviewed in refs. 1,7,12; see also refs. 13-15 in a special issue of the Journal of Experimental Zoology, entitled "Environmental sex determination in reptiles: patterns and processes"). Many of these publications deal with ecological and/or evolutionary aspects (adaptive significance) of TSD. Recently, an appreciable progress has been made in our understanding of the mechanisms, chiefly the implication of steroids in TSD. Thus, in this paper, after having briefly described the different patterns of TSD and their features (thermosensitive periods, transitional ranges of temperature and pivotal temperatures), we focus on the role of steroids and enzymes of steroidogenesis (mainly estrogens and aromatase). Then, we propose working hypotheses taking into account all data obtained so far for the possible molecular mechanisms of TSD. Finally, we will examine how these mechanisms become integrated into the recent data obtained in other vertebrates.

Patterns of temperature-dependent sex determination (TSD)

Three TSD patterns have been generally recognized from artificial incubations of eggs at constant temperature (ref. 16, and figure 1):
* Pattern Ia : low temperatures yield 100% (or predominantly) males, high temperatures yield 100% (or predominantly) females.
* Pattern Ib : low temperatures yield 100% (or predominantly) females, high temperatures yield 100% (or predominantly) males.
* Pattern II: low and high temperatures yield 100% (or predominantly) females, intermediate temperatures yielding various ratios of males.

Pattern II occurs in the three groups of reptiles known to exhibit TSD (crocodilians, turtles and lizards). Therefore, it may be ancestral, and patterns Ia and Ib may be derived from it (figure 1) through loss of feminization at low or high temperatures, respectively[17] or because these temperatures, maintained constantly, are beyond the limits of survival of embryos. Besides pattern II, pattern Ia has also been found in turtles[12,15,16]. Pattern Ib was originally described in two lizards, *Agama agama*[8] and *Eublepharis macularius*[1] and in the alligator *Alligator mississippiensis*[18]. However, recent studies looking for the effects of larger ranges of temperatures have shown that pattern II really occurs both in *E. macularius*[13] and in *A. mississippiensis*[14]. Studies at warmer temperatures than those previously used might well yield predominantly females in *A. agama*, too[15]. Half of the 22 extant species of crocodilians have been examined for occurrence of TSD. All exhibit this phenomenon and 5 of them, examined in detail, display pattern II[14]. Recent data in several species of lizards indicate that this pattern would be also widespread, or even unique, in lizards[13]. If these indications were confirmed by further studies, thus TSD in reptiles would obey only pattern II in crocodilians and lizards and both pattern Ia and pattern II in turtles (figure 1).

In each TSD pattern, both sexes and sometimes intersexes are obtained in the transitional range(s) of temperature (TRT1, TRT2, figure 1) between male-producing and female-producing temperatures. Within TRT, the pivotal temperature has been operationally defined as that temperature during incubation at constant temperature which gives 50% individuals of each sexual phenotype[19]. In patterns Ia and Ib, there is one pivotal temperature whereas in pattern II, there are two pivotal temperatures (P1 and P2, figure 1). Pivotal temperature can be determined for a single clutch, a population or a species. Determination of pivotal temperature for a population requires the incubation of several clutches since inter-clutch variability can be important (>1deg.C)[19]. For a species, geographic variation in pivotal temperature would be also considered[15].

The transitional range of temperature appears to be narrow in some species (for example, less than 1deg.C in the marine

turtle *Dermochelys coriacea*), and much broader in other species (about 4deg.C in *Chelonia mydas*, another marine turtle). Within this range, the heritability of the zygotic character of sex ratio is high and reveals a genetic component of sex determination (see discussion in ref. 20). As suggested in *Emys orbicularis*, this component could correspond to an underlying classical mechanism of genotypic sex determination which is operational in incubations around the pivotal temperature(s) and probably also in nature[21,22].

The thermosensitive periods (TSPs) for gonadal differentiation have been determined in a few species of crocodilians and turtles and in a lizard, using shifts of temperature from male-producing to female-producing temperatures and *vice versa*. These periods vary with experimental procedures (mainly the temperatures chosen) and with the criteria used to define the window of sensitivity to incubation temperatures[19]. However, it can be stated that TSPs extend for periods corresponding to 18-30% of embryonic development, in the second quarter (lizard), middle third (turtles) or third quarter (crocodilians) of the incubation period. In all cases, TSP corresponds to the first steps of sexual differentiation of the gonads[14,23,24].

Involvement of steroids in gonadal differentiation

In the turtle *Emys orbicularis*, the incubation of eggs around the pivotal temperature yields females with typical ovaries and males with typical testes or testes with an ovarian-like cortex presenting various degrees of development[12]. This shows that gonadal differentiation depends on the dosage of masculinizing or feminizing substances. These substances were expected to be steroid hormones since treatments with androgens or estrogens were known to modify or even reverse the gonadal differentiation in all vertebrates except eutherian mammals. Moreover, it was expected that endogenous steroid synthesis in the gonads was influenced by the incubation temperature in reptiles with TSD.

1) Evidence of early and temperature-dependent steroid synthesis in the gonads

Gonadal steroid synthesis was studied in *Emys orbicularis* embryos incubated at a male-producing or at a female-producing temperature, during (first third) and after the thermosensitive period[25]. At each stage, pools of explanted gonads were able to metabolize tritiated pregnenolone, progesterone, dehydroepiandrosterone, or androstenedione used as precursors, showing the presence and activity of enzymes involved in steroidogenesis. The major steroidogenic pathways deduced from this study are shown in figure 2. The enzymes 5alpha-reductase and 20B- hydroxysteroid oxidoreductase had the highest activities at both temperatures. The activity of 3B-hydroxysteroid dehydrogenase-5-ene-4-ene isomerase (3B-HSD) was higher at male-producing temperature than at female-producing temperature[25]. This higher 3B-HSD activity in differentiating testes has also been found in two other turtle species (*Testudo graeca*, *Dermochelys coriacea*) and in the alligator, *Alligator mississippiensis* (reviewed in ref. 26). Its biological significance remains unknown.

The metabolism of androstenedione in gonads of *Emys orbicularis* resulted in very low amounts of estrogens[25]. However, measurement by a sensitive radioimmunoassay of the gonadal endogenous content of estrogens showed that these hormones were at higher levels in differentiating ovaries than in differentiating testes during the early stages of the thermosensitive period[27].

2) Evidence of the implication of estrogens in gonadal differentiation

Although gonadal estrogens are produced in low amounts, they have been shown to play a major role in gonadal differentiation as a function of temperature. Exogenous estrogens, injected into eggs or applied onto the eggshell before or during the thermosensitive period, induced ovarian differentiation at male-producing temperatures in species of the three groups of reptiles exhibiting TSD (reviewed in ref. 26). Specific binding of tritiated estradiol by the gonads was found during the thermosensitive period, and was similar at both male-producing and female-producing temperatures, in *Crocodylus porosus*[28]. Moreover, in several species the period of estrogen sensitivity was shown to parallel the period of temperature sensitivity, and female-producing temperature and estrogens were shown to exert a synergistic effect on gonadal differentiation (reviewed in ref. 26).

In *Emys orbicularis* embryos, treatments by an antiestrogen (tamoxifen) or by nonsteroidal aromatase inhibitors (CGS 16949A, CGS 20267) resulted in various degrees of masculinization of gonads at female-producing temperature[27,29]. Tamoxifen competes with estrogens at the level of estrogen receptors. It has been reported to have both antagonistic and agonistic effects in mammalian tissues. Both effects were also obtained on gonadal differentiation in *Emys orbicularis*. Indeed, testicular cords differentiated in the medulla (antagonistic effect), but an ovarian-like cortex was maintained at the surface (agonistic effect); gonads were ovotestes[27]. In other reptilian species, the antagonistic effect of tamoxifen was not observed[26].

Aromatase inhibitors prevent the conversion of androgens to estrogens. In *Emys orbicularis*, gonads of embryos incubated at female-producing temperature treated by these inhibitors were either typical testes, ovotestes or masculinized ovaries depending on the degree of inhibition of endogenous estrogen production[29]. In *Trachemys scripta*, another turtle species with TSD, treatment by CGS 16949A at both pivotal and female-producing temperatures and with CGS 20267 at female-producing temperature increased the ratio of males[30,31]. Likewise, in *Chelydra serpentina*, treatment by CGS 16949A increased the percentage of males at predominantly female-producing temperature [32].

Altogether, results of these experiments show that testicular cord differentiation (Sertolian epithelium including germ cells) and ovarian cortex differentiation (multiplication of germ cells in the surface epithelium and their entering in meiosis) are controlled by estrogens. At low levels of estrogens (masculinizing temperature), testicular cords differentiate and cortex does not develop or is reduced; at higher levels of estrogens (female-producing temperature), the development of testicular cords is inhibited and that of ovarian cortex is stimulated[27].

3) Effects of androgens on gonadal differentiation

The effects of two androgens, testosterone and its reduced derivative dihydrotestosterone (DHT), were examined (testosterone is converted to 5alpha-DHT or 5B-DHT through the activity of 5alpha-reductase or 5B-reductase). In turtle embryos (*Emys orbicularis*, *Trachemys scripta*), testosterone treatments had no masculinizing effect at female-producing temperature but induced, at male-producing temperature, a partial (cortex development and maintenance of testicular cords) or complete feminization of gonads. This "paradoxical" effect was explained by the aromatization of testosterone to estradiol[12,33]. Indeed, it was not observed with DHT (5alpha-?) which is not aromatizable[31,33].

In *Trachemys picta*[33] and *Alligator mississippiensis*[34], exogenous DHT could not overcome the effects of a 100% female-producing temperature. Moreover, in *T. picta*, reductase inhibitors had no effect on gonadal differentiation at a 100% male-producing temperature[33]. In our opinion, these results exclude a major role of androgens compared to that of estrogens. In particular, the implication of androgens, via androgen receptors, in the differentiation of testicular cords is improbable. However, results of a series of experiments performed in *Trachemys scripta* would indicate that DHT favours male differentiation of gonads around the pivotal temperature. Treatment with DHT increased the ratio of males, whereas treatment with reductase inhibitors increased the ratio of females in incubation at pivotal temperature. Moreover, simultaneous administration of DHT and estradiol resulted in the formation of ovotestes; such gonads are frequent in *Emys orbicularis*, but have not been observed normally at pivotal temperature in *Trachemys scripta*[33]. These results could be explained by a competition of DHT with estrogens at the level of estrogen receptors or/and by a synergistic action of the androgen and the anti-Müllerian hormone (see below).

Possible molecular mechanisms of temperature-dependent sex determination

1) A key role for aromatase

Estrogens (estrone and estradiol-17B) are synthesized by aromatization of androgens (androstenedione and testosterone). This conversion is catalyzed by the enzyme aromatase cytochrome P 450 (P 450 arom), known as aromatase.

Gonadal aromatase activity was measured in embryos of *Emys orbicularis* and *Dermochelys coriacea* from the beginning of the thermosensitive period up to hatching. In both species, it was very low at the beginning of the thermosensitive period. Then, it increased exponentially in differentiating ovaries and decreased slightly around hatching, whereas it remained very low in differentiating testes up to hatching[35,36]. Moreover, a high concordance between the thermosensitive periods for both ovary differentiation and increase in aromatase activity was found[36,37]. In embryos of *Crocodylus porosus* and *Alligator mississipiensis*, aromatase activity increased strongly in the gonad-adrenal-mesonephric complex during ovarian differentiation at female-producing temperature. In female hatchlings, most aromatase activity was found to be in the ovary while in males, the very low levels were similar in both the testis and the adrenal-mesonephros. These results are thus very similar to those found in turtles[38,39].

Finally, a good concordance between gonadal aromatase activity and gonadal structure was established in *Emys orbicularis* embryos obtained from eggs incubated in different conditions: at pivotal temperature, at 30deg.C and treated by an aromatase inhibitor, or shifted from a male-producing to a female-producing temperature or *vice versa* during the thermosensitive period. In all cases, ovaries, testes and gonads with various degrees of intersexuality were obtained. These different structures were found to be well correlated with aromatase activity and consequently with estrogen content: the higher the endogenous estrogen synthesis, the greater the feminization of gonads (ref. [37] and unpublished results). In this process, the regulation of the expression of aromatase itself plays the key role and would be temperature sensitive[37]. Since, in *Emys orbicularis*, the temperature at which the aromatase assays were carried out had no

significant effect on the enzyme activity, it was concluded that temperature did not act directly on the activity of the enzyme but would act, directly or indirectly, on its synthesis[35,37]. Assays in *Alligator mississipiensis* led to the same conclusion[39].

2) Working-hypotheses

A model attempting to integrate data obtained with estrogens, antiestrogens, androgens, aromatase inhibitors and reductase inhibitors has been proposed recently. In this model, incubation temperature would activate at least four genes: the genes encoding for aromatase and for estrogen receptor at female-producing temperature, and the genes encoding for reductase (5alpha-?, 5B-?) and androgen receptor at male-producing temperature[33]. In our opinion, such a model is too complicated to account for the evolution of TSD. Indeed, cladistic analysis of reptilian phylogeny shows that TSD (and/or GSD) has evolved multiple times in reptiles[7]. From an evolutionary point of view[40], a model considering only one ubiquitous target for temperature would be parsimonious.

As seen above, the involvement of estrogens and the key role for aromatase in gonadal differentiation are irrefutable. Thus, the model presented here (figure 3) is based on the possible mechanisms of temperature regulation of expression of the aromatase gene at male- and female-producing temperatures. It derives from a more general model postulating involvement of aromatase in the sexual differentiation of gonads in vertebrates[4]. The regulation of transcription of the aromatase gene is multifactorial. Some factors such as (or *via*) cyclic AMP activate its transcription, other factors such as the anti-Müllerian hormone (AMH, produced by Sertoli cells of testicular cords) repress it[4,26]. Moreover, to explain the exponential increase in aromatase activity in differentiating ovaries, a positive feedback by estrogens amplifying the activation of the aromatase gene can be expected. Temperature could activate or repress transcription of the aromatase gene through three distinct ways (figure 3), one of them being necessary and sufficient to account for male or female gonadal differentiation:

Way no. 1: temperature regulates a thermosensitive factor which is directly or indirectly (*via* cyclic AMP or another factor) involved in the activation of transcription of the aromatase gene; a simple hypothesis is that a thermosensitive factor would be implicated in the formation of the transcriptional initiation complex of the aromatase gene itself[37].

Way no. 2: temperature regulates a thermosensitive factor which is directly or indirectly involved in the activation of transcription of a gene encoding for a factor repressing aromatase; for example, a thermosensitive factor would be implicated in the formation of the transcriptional initiation complex of the AMH gene.

Way no. 3: temperature regulates the expression of heat shock protein(s) (hsp) involved in the binding of estrogens to estrogen receptors, or is implicated in the dissociation of hsp(s) from the complex estrogen-estrogen receptor which is activated.

In both ways 1 and 2, temperature acts on a factor implicated in the regulation of the aromatase gene itself, while in way 3, temperature acts on a factor (hsp) implicated in the formation or activation of the complex estrogen-estrogen receptor. A basal level of estrogens, amplified at female-producing temperature by the positive feedback control of these hormones, is thus required in way 3.

The model considers a possible role for DHT *via* 5alpha-, or 5B-, reductase. This role appears to be restricted to incubations around the pivotal temperature. However, it cannot be neglected, since such conditions probably often occur in nature[22]. Testosterone can be converted either to estradiol-17B (through aromatase activity) or to 5alpha- or 5B-dihydrotestosterone (through 5alpha- or 5B-reductase), or to both metabolites. Therefore, slight synthesis of DHT at female-producing temperature could be simply the consequence of high aromatase activity, whereas higher synthesis of DHT at male-producing temperature could result from low aromatase activity. Study of the metabolism of substrates other than DHT in *Emys orbicularis* and *Dermochelys coriacea* has shown that 5alpha-reductase activity (in *E. orbicularis*, figure 2) or both 5alpha- and 5B-reductase activities (in *D. coriacea*) are relatively high and similar at both male-producing and female-producing temperatures (ref. 25, and unpublished results). These results exclude a temperature regulation of the gene encoding for these enzymes. A possible explanation for the masculinizing effect of DHT around the pivotal temperature is that DHT might compete with estrogens at the estrogen receptor level and/or exert a synergistic action with AMH as suggested by experiments carried out in birds[41].

Either of the three possible ways for regulation of aromatase leads to increasing synthesis of estrogens when transcription of the aromatase gene is activated (at female-producing temperature) and to very low synthesis of estrogens when transcription of the aromatase gene is repressed (at male-producing temperature).

Structural genes for both ovarian and testicular differentiation are present in all individuals. High endogenous levels of estrogens during the thermosensitive period activate ovarian differentiation genes and repress testicular differentiation

genes: gonads differentiate as ovaries. With low amounts of estrogens, ovarian differentiation genes are not activated and testicular differentiation genes are not repressed: gonads differentiate as testes[4]. Thus, in our model, testicular differentiation is constitutive.

Is there a favoured way among the three ones proposed for the regulation of expression of the aromatase gene ? Way 3 is seductive because it implicates ubiquitous protein(s) (hsp[s]) that could explain why TSD appeared independently multiple times in reptiles. Two data argue however against it: estrogen receptors are present and able to bind estrogens at both male-producing and female-producing temperatures[28]; binding of the estrogen-estrogen receptor complexes to the estrogen response elements of the DNA is apparently normal at male-producing temperature[27]. However, in the experimental procedures of feminization, estrogens are always in excess and probably in higher amounts in gonads than in natural conditions. High gonadal concentrations of hormones might enhance their binding to estrogen receptors at male-producing temperature.

The regulation of expression of the AMH gene (way 2) is an attractive hypothesis, since this factor is probably involved in the regulation of aromatase in mammalian gonads. However, Müllerian ducts were regressing in *Emys orbicularis* embryos shifted from a male-producing temperature to a female-producing temperature at the early stages of the thermosensitive period[23] and in embryos incubated at a female-producing temperature and masculinized by aromatase inhibitors[29]. Thus, the synthesis of AMH is not temperature-dependent.

At present, there is no argument against or in favour of way 1.

A partial single copy P450 arom from a turtle (*Malaclemys terrapin*) has been cloned using a cDNA library constructed from ovarian mRNA. This partial clone is highly homologous to other vertebrate aromatases[42]. In further studies, it will be of great interest to examine the promoter region(s) for the P 450 arom gene in reptiles with TSD in order to determine potential regulatory elements of this gene and compare them to those in other vertebrates[37,42].

Comparison with other vertebrates, conclusions

The involvement of sex steroids in gonadal differentiation is now well documented in all classes of non mammalian vertebrates. Data have shown the implication of estrogens and androgens in fishes, estrogens and possibly androgens in amphibians, and estrogens in reptiles and birds (reviewed in ref. 4; see also discussions in refs. 27,29). The feminizing effects on gonadal differentiation by exogenous estrogens have been shown *in vivo* in marsupial mammals but not in eutherian mammals. Recent findings in human and mice argue rather against the implication of estrogens in gonadal differentiation, while *in vitro* experiments on gonads of rat embryos suggest a possible role for these hormones. In human, ovaries were found in a patient presenting a syndrome of aromatase deficiency[43]. In mice, ovaries were present in females of a mutant line without a functional estrogen receptor[44]. However, a residual activity was found in the first case and a residual estrogen binding was detected in the second case. Thus, it cannot be excluded that the low levels of estrogen production or estrogen binding were sufficient to induce ovarian differentiation in embryos. *In vitro*, AMH induced the differentiation of testicular cord-like structures in gonads of genotypic female rat embryos thus reproducing the freemartin effect[45]. Moreover, AMH repressed aromatase synthesis[46]. Therefore, the masculinization of gonads by this factor could result from the decrease in the level of gonadal endogenous estrogens[4,27]. Altogether, these results suggest that a mechanism implicating estrogens has been conserved, at least partly, in the gonadal differentiation of all vertebrates. In this mechanism, the expression of aromatase plays a key role, but its regulation, multifactorial, probably varies in the different systems of sex determination. In both XX/XY and ZZ/ZW GSD systems, the control of aromatase regulation is strictly genetic, whereas in TSD the effect of temperature is superimposed upon a genetic program[4].

There are several known or putative regulating factors of aromatase in embryonic gonads:
- cyclic AMP, which activates the transcription of aromatase, probably in all vertebrates;
- AMH, which represses the transcription of ovarian aromatase in reptiles, birds and mammals[47];
- SF-1 (Steroidogenic Factor 1), an orphan nuclear receptor, regulating steroidogenic P 450 genes including P 450 arom and the AMH gene in mammals[48,49];
- SRY, the probable Testis Determining Factor in mammals, which has been shown to bind *in vitro* to the promoter regions of the human AMH and aromatase genes[50].

Mc Elreavy *et al.*[51] have suggested that SRY in mammals would act as a repressor of a negative regulator of testicular development. Moreover, the recent discovery of a duplicated locus (Dosage Sensitive Sex Reversal, DSS) on the X chromosome, responsible for sex reversal in human individuals with a 46, XY karyotype[52], suggests that SRY would act as a repressor of ovarian development, allowing thus testis differentiation. In our previous model[4] and the model presented here, aromatase (the activity of which results in estrogen synthesis) can be seen as both a negative regulator of

testicular development and an activator of ovarian development. These models accord with the Wilkins hypothesis(53) on the evolutionary origin of the negative regulatory cascades of genetic sex determination.

As shown above, cyclic AMP, AMH and a thermosensitive factor probably intervene in the regulation of transcription of P 450 arom gene in reptiles with TSD. SF-1 or an homologue has not yet been looked for, but probably exists, since it appears to act at multiple levels of the reproductive axis(54) and therefore would be conserved. Paralogous SRY genes have been cloned in *Alligator mississippiensis*(3) and *Chelydra serpentina*(55) but, no SRY homologue has been found in non mammalian vertebrates(56).

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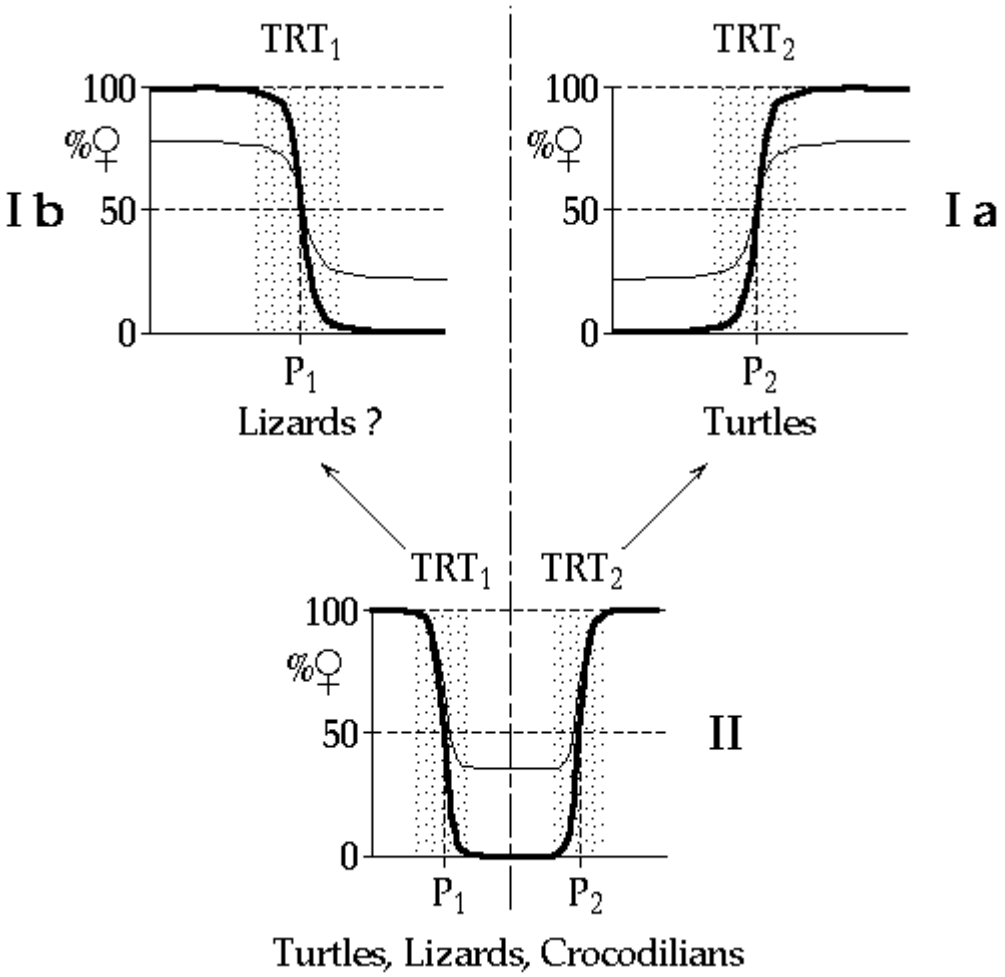

Figure 1: Patterns of Temperature-dependent Sex Determination (TSD) in reptiles. In pattern II, there are two transitional ranges of temperature (TRT1 and TRT2) and two pivotal temperatures (P1 and P2), whereas in patterns Ia and Ib only one transitional range of temperature and one pivotal temperature are observed. Ia and Ib could derive from II (FT, female-producing temperature; MT, male-producing temperature).

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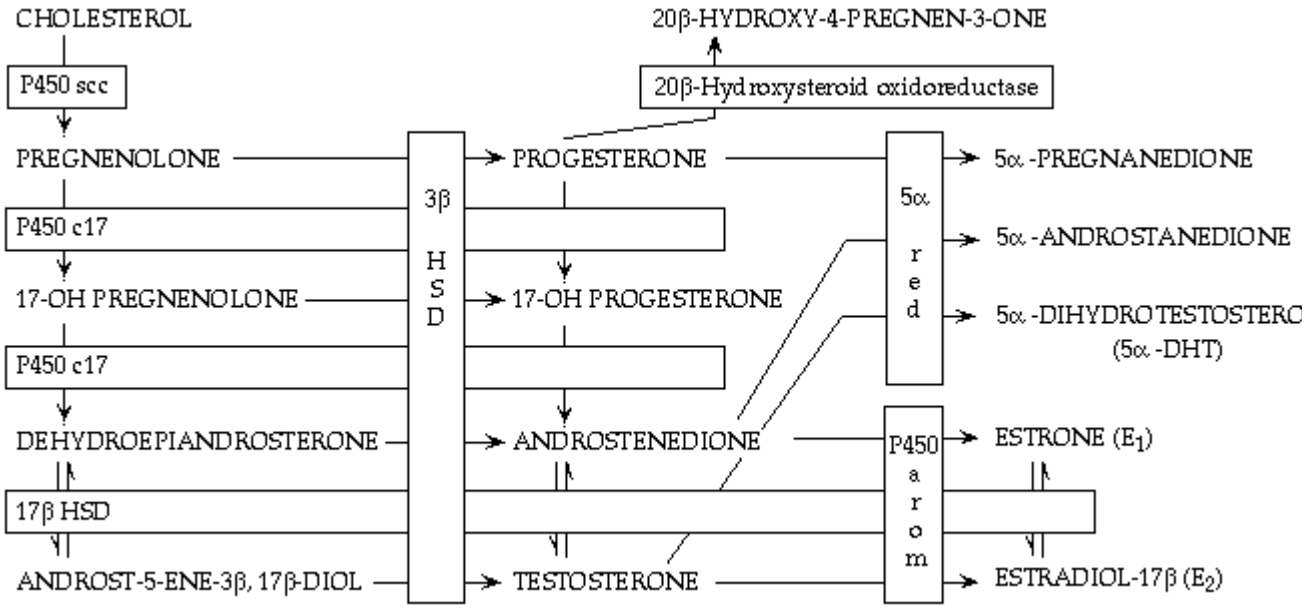

Figure 2: Schematic representation of the major steroidogenic pathways in the embryonic gonads of the European freshwater turtle *Emys orbicularis*. (P 450 scc, cholesterol side-chain-cleavage cytochrome P 450; P 450 c17, cytochrome P 450 enzyme catalyzing both 17alpha-hydroxylase and 17-20-lyase activities; P 450 arom, aromatase cytochrome P 450; 3B-HSD, 3B-hydroxysteroid dehydrogenase -5-ene-4-ene isomerase; 17B-HSD, 17B-hydroxysteroid dehydrogenase; 5alpha-red, 5alpha-reductase) (modified from 25).

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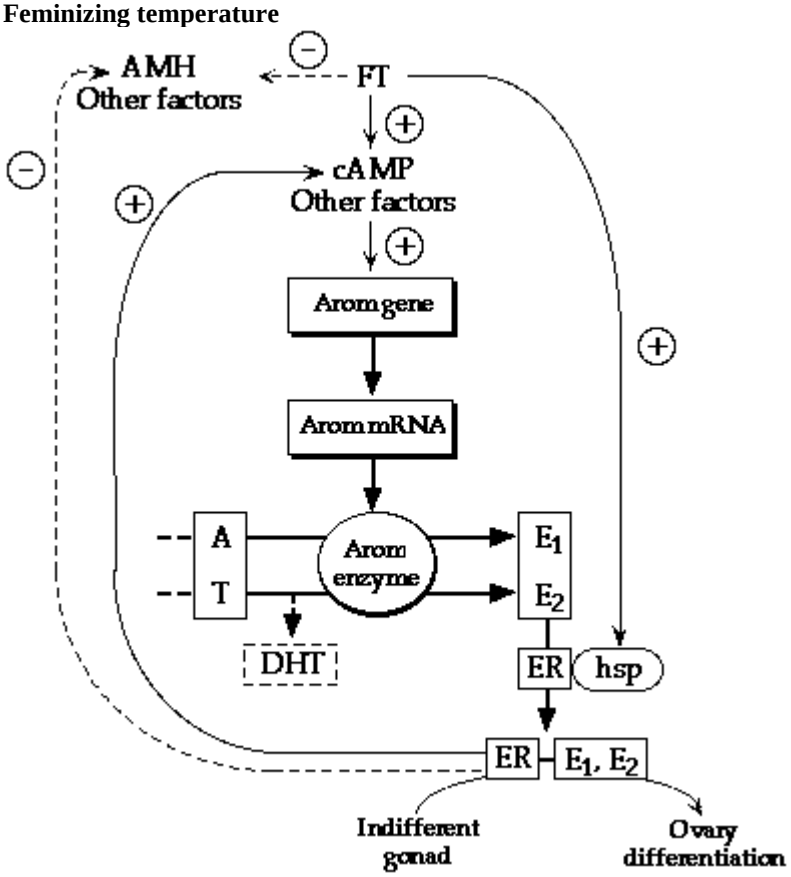
Feminizing temperature



Masculinizing temperature

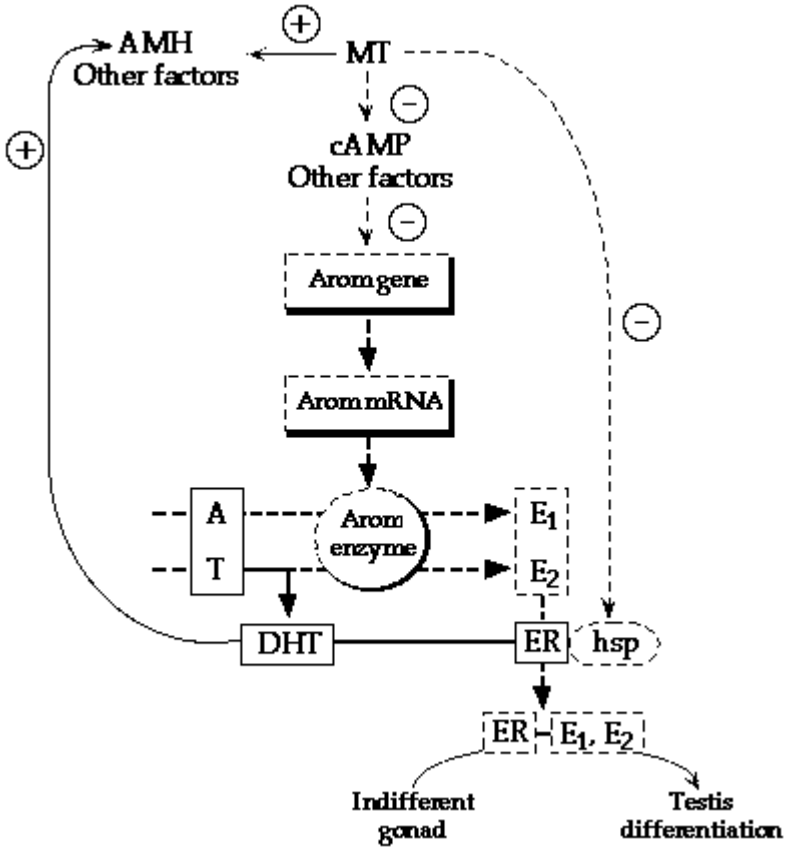


Figure 3: Possible molecular mechanisms of the action of temperature at male- and female-producing temperatures in reptiles with TSD. (1, 2, 3: the three possible ways for the thermosensitive regulation of transcription of the aromatase gene; , activation; , repression; FT, female-producing temperature; MT, male-producing temperature; AMH, anti-Müllerian hormone; A, androstenedione; E1, estrone; E2, estradiol-17B; ER, estrogen receptor; T, testosterone; DHT, dihydrotestosterone; hsp, heat-shock protein).

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