Abstract in general adrenal glands and gonads play

AbstractIn general adrenal glands and gonads play a veryimportant role in sex differentiation and steroidogenesis. These two aresystems are closely related as they share a common region of origin i.

e. mesoderm and both are involved in steroidogenesis. Various biological eventsoccur during adrenal and gonadal steroidogenesis. In this article important signaling pathways and transcription factorsinvolved in regulation of steroidogenesis and adrenal growth have beensummarized. Present review illustrates various novel signaling pathways suchWnt, Sonic hedgehog, Notch, ?-catenin involved in adrenal gland morphology andits functions that are deeply interconnected. Certain nuclear receptor such asSteroidogenic Factor-1 acts as critical regulator of development andhomeostasis of the adrenal cortex and gonads. SF-1 is a nuclear receptoralmost exclusively expressed in the steroidogenic tissues of the hypothalamicpituitary-adrenal/gonadal axis. Mitogen-activated protein kinases areserine/threonine kinases involved in the expression of the Steroidogenic acuteregulatory protein and steroidogenesis.

Characterization of proteins that areencoded by amh, dax1 and cyp19a1 which play important roles ingonad differentiation and to evaluate the relation between gonadal expressionof Fushi tarazu factor-1, StAR andcytochrome P450-11A in reproductive maturation process. This article aimed to describe the various signaling mechanismsand novel transcription factors involved at genomic level in common to adrenaland gonadal development in fishes and lower vertebrates. Keywords: Gonadal development; Sex differentiation; Adrenal growth; Steroidogenic Factor-1; Steroidogenic acute regulatory protein; Mitogen-activated

proteinkinases; Introduction Steroidogenesis involves the synthesis of steroidhormones that are derivatives of cholesterol which are synthesized by varioustissues, most prominently the adrenal gland and gonads. These are usually found inchordates and arthropods. Fishes, for exampleteleosts, produce several types of bioactive gonadal steroids, includingprogestogens, estrogens, androgens and various derivatives of steroids.

Steroids are required for development, maintenance, homeostasis andreproduction. Steroids direct the development of germ cells and accessory glands andorgans, as well as the modification of the behaviour, to ensure that sexualreproduction can take place In adult vertebrates, these steroids are produced at appropriatetimes in specialized steroid producing cells called gonads. These cellsexpress a group of steroidogenic enzyme genes whose products modify itscholesterol and derivatives 1.

Although many steroids are identicalchemically in all major vertebrate classes, the role of these steroids maydiffer. However, unique steroid hormones have evolved in some vertebrateclasses, especially amongst fishes, to fulfil particular functions. Sex differentiation is initiated andcontrolled by gonadal steroid hormones.

These hormones performeddifferent functions and permanently differentiated into sex organs duringdevelopment. Steroid hormones are synthesized in steroidogenic cells of theovary testis and brain that are required for normal reproductive function and bodily homeostasis. Steroidogenic endocrinetissues such as the adrenal and the gonads respond to trophic hormones andother external stimuli with rapid surge in steroid

hormone production 2. Theacute and chronic regulation of steroidogenesis is controlled by trophichormones that usually occur onorder of minutes and hours, respectively. Chronic regulation of steroidogenesisby LH or ACTH occurs at the level of gene transcription 3. The acute response is initiated by themobilization and delivery of the substrate for all steroid hormone biosynthesis. Cholesterol, from the outer tothe inner mitochondrial membrane, where, it is metabolized to the pregnenoloneby the cytochrome P450 cholesterol side chain cleavage enzyme (p450scc).

The Steroidogenic acute regulatory(StAR) protein is the one which regulates the true rate-limiting step in steroid biosynthesis, i. e. thedelivery of cholesterol from the outer to the inner mitochondrial membrane 4. The central role of StAR was proven by twoobservations by robust steroid hormone synthesis followed co-transfection ofStAR and the cholesterol side-chain cleavage system into nonsteroidogenic COS-1cells 4, 5. Second, patients with StAR mutations havecongenital lipoid adrenal hyperplasia, in which all adrenal and gonadalsteroidogenesis was disrupted 5, 6.

The expression of this protein ispredominantly regulated by cAMP-dependent mechanism in the adrenal and gonads. Gonadal developmentVertebrate reproduction depends on the function oftwo distinct gametes, sperm and eggs, which develop into different organs, thetestis and the ovary. These are composed of germ cells, supporting cells andinterstitial cells. The ovary and the testis are essential for gametogenesis. Amature ovary consists of an ovarian cavity, the germinal or surface epithelium, and the stromal compartment.

In fishes such as teleosts, germ line stem cellsand mitotically active oogonia reside in the germinal epithelium. Thisstructure is similar to the surface epithelium in mammals. Follicles arepresent in the stromal compartment where oocytes grow and steroid hormones areproduced.

In the testis, spermatogenesis from germ line stem cells to spermoccurs in tubules or lobules, and the interstitial tissue that produce steroidhormones resides between these structures. The two reproductive organs aregrossly different, but they both are composed of developmentally common celllineages, supporting cells, interstitial cells and germ cells. Germ cells, critical for conveying the genetic information to the next generation, are veryspecial in that they are segregated from other cells at a very earlydevelopmental stage when major positional information is being established, andmigrate into the future gonadal area. Early stagegerm cells that have not reached the gonad are called primordial germ cells(PGCs). PGCs in teleosts are morphologically identified and functionallyspecified by the allocation of cytoplasmic determinants that includesRNA-binding proteins NANOS, VASA and TUDOR which are localized on granule-likestructures or nuage 7. This finding was similar to which has been observedin other lower vertebrates and Drosophila. In some fish such as medaka, nanos3was found to be the earliest marker so far examined for germ cells, and usingthis marker, PGCs was first identified at an early gastrulation stage 8. The migration of PGCs consists of at least threemechanically distinct modes 8, 9.

First, at an early gastrulation stage, PGCsactively migrate towards the marginal zone, a process which depends on thechemokine receptor CXCR4 and its ligand, SDF1A. Second, at the lategastrulation and early

somitogenesis stages, PGC movement depends on theconvergent movement of somatic cells. Third, after aligning bilaterally, PGCs, governed by interactions between CXCR4 and SDF1B, resume active and directionalmigration towards the posterior end of the lateral plate mesoderm, wheregonadal somatic precursors arise 10. In teleosts, Sertoli and granulosa cells weresuggested to share a common origin, namely, the supporting cells expressing thesox9b gene in the bipotential gonadal primordia. Interestingly, sox9bexpression is found in both XX and XY supporting cells 11, an observation that is in sharp contrast to the situation in mammals, wheresox9 is only expressed in Sertoli cells and is both required and sufficient fortesticular development 12, 13. The sox9b expressing cells begin to express dmrt1, an indication ofdifferentiation into Sertoli cells, and are mainly located inside the lobulesof the adult testis as gonadal primordia develop into ovarian structures, thesox9b -expressing cells constitute germline stem cell niches, or germinalcradles.

As mentioned above, early oogenesis, from germ line stem cells toearly diplotene oocytes, proceeds in the cradles. Subsequently the diploteneoocytes and the surrounding somatic cells exit from the germinal cradle andrecruit theca cells to form follicles. As a result, the follicles in thestromal compartment have 2 layers of somatic cells, outer theca cells and innergranulosa cells. During this step, the granulosa cells lose sox9b expressionwhile foxl2, a marker of granulosa cells, is activated 11, 14suggesting granulosa cells originate from the sox9b -expressing cells. Bothfollicular formation and oocyte exit from germinal cradles appears to dependupon a series of successive processes 14. Histological analysis of

other teleost fish also supports this observation 15, 16. Interestingly, in the testis, sox9b expression is very intense in the Sertolicells located most distally in the lobules. The distal region is predominatelyoccupied by the most undifferentiated type of germ cells, type A spermatogonia.

In the ovary, sox9b isexpressed in the germinal cradles representing niche regions. Collectively, these observations suggest that the common function of sox9b -expressing cellsmay be the maintenance of stem-type germ cells during early gametogenesis. During testicular development, steroidogenic genesrequired for the production of steroid hormone(s), e. g.

p450scc/cyp11a1 andhsd3b, begin to be expressed inpresumptive Leydig cells located in the marginal regions of the lobuleSteroidogenic genes are expressed in ftz-f1 -expressing cells during testiculardevelopment 17. This suggests that ftzf1 regulates a set of steroidogenic genes and thatandrogen production may occur in a single cell lineage of ftz-f1 – expressingcells. In rainbow trout, immunohistochemical analysis also revealed thatP45011B/CYP11B, P450scc/ CYP11A1, HSD3B, and P450c17/CYP17A1 were allco-localized in interstitial Leydig cells 18. By contrast, during ovarian development, at least 2 types of theca cells seemto be present in medaka. Some fishes express only aromatase. Expressionanalysis using aromatase-reporter transgenic medaka fish has revealed p450c17 and aromatase were exclusivelyexpressed 19. These results suggest that theca cells may be derived from at least twodistinct populations in medaka. Alternatively, the two types of theca cells mayshare a common precursor that expresses the ftz-f1 gene, and that generatesoffspring capable of either maintaining or down regulating ftz-f1 expressionand initiating aromatase expression 20.

AdrenaldevelopmentInvertebrates, adrenal glands composed of two distinct parts, outer adrenalcortex and inner adrenal medulla. Adrenal cortex secretes three major hormonesglucocorticoids, mineralocarticoids and adrenal androgens. Adrenal androgens involvedin the gender differentiation in human beings mainly dehydroepiandrosterone(DHEA) and testosterone.

Cellular organization of gonads is similar in allvertebrates, based on different progression can trigger bipotential gonads, forms either ovaries or testis. Gonads are originated from thickening of theventrolateral surface of the embryonic mesonephros called the genital ridge. Theclassic experiment of Jost 21demonstrated that female differentiation occurs irrespective of the genetic sexin the absence of testicular hormones. Previous expression data suggestedthat GATA4 was involved in sex determination 22, 23and in vitro data suggested a role for GATA4 in the regulation of genesexpressed in the gonads downstream of Sry, including Mis, inhibin ?, and steroidogenic acute regulatory protein (StAR) (reviewed by 24, 25.

Adrenal steroid hormones are effective in differentadaptive responses in the internal and external environment stress ofvertebrates. The sex determination region of Y chromosome (SRY) gene requiredto initiate signaling for male gonadal differentiation. Many other genesinvolved in gonoadogenesis are GATA4 and FOG2 26. Mammalian gonads arise in both sexes from bilateral genital ridge that have thepotential to develop as ovaries or testes 27, 28. In humans gonadal differentiation occurs from the 10th through 12thembryonic week.

Steroidogenic factor 1 (SF-1) transcription factorcritical for adrenocortical development and homeostasis. SF1 is also known asadrenal four-binding protein or nuclear hormone receptor Ad4BP, encoded by thegene NR5A1. All cells that belong to steroidogenic lineages of the adrenal andgonads express SF1, including subpopulations of long-term retained progenitorcells in each organ 29, 30. Therefore, SF1 expression defines the identity ofthese cells and commitment to steroidogenic differentiation 31-33. The expression of SF1 is detectable early in fetallife, between the AGP formation and the ultimate establishment of the adrenalprimordium 30. Genetic loss of Nr5a1 or its upstreamtranscriptional regulators Pbx1, Wt1, and Cited2, interferes with AGP formationleading to various degrees of adrenal hypoplasia in mice 34-36. While Nr5a1 is continuously expressed from thetime of adrenal primordium formation throughout the adult life, duringembryonic stages and early fetal life in mice, the Nr5a1 expression is drivenby the fetal adrenal-specific enhancer (FAdE), which becomes inactive when thedefinitive cortex forms, suggesting that distinct mechanisms sustain Nr5a1expression in the fetal and in the definitive cortex .

Genes essential forearly gonadal development: Acquisition of sexual dimorphic phenotype conditionis an important role in mammalian gonadal development. In this absence orpresence of Y chromosome at fertilization embryonic gonads differentiate in toeither ovaries or testis. Major four genes are known to be required for development ofbipotential gonads (a) the orphan nuclear receptor Steroidogenic factor-1 (SF1 orFtz-F1) 37 (b) Wilms tumor associated gene (WT1; 38, a zinc fingerDNA–binding protein, (c) Lhx1 (also known as Lim1), and (d) Lhx9, two LIM classhomeobox proteins 39.

SRY, SF-1, Wilms’ tumor related 1(WT1), GATA4, and SOX9, were emerging models thatsuggest complex interactions among these genes in gonadal development (KeithParker). SRY is the critical initiator oftestis development is a gene located immediately adjacent to the pseudo autosomalregion of the short arm of the Y chromosome, designated SRY for Sex-determiningRegion-Y chromosome. SRY has been identified as the testis-determining factor(TDF), the key gene responsible for testis development in XY embryos. Once thegonads are formed, the pivotal event in male sexual differentiation isexpression of the SRY gene. SRY is necessary andsufficient to initiate the male development cascade 40.

In the absence of Sry, or if Sry isexpressed at insufficient levels, the support cell precursors differentiate asgranulosa cells, thus initiating the ovarian pathway 26. The molecular mechanisms upstream and downstreamregulations of Sry are not well understood. Trivosian et al. 26, demonstrated that the transcription factor GATA4 and itsco-factor FOG2 are required for gonadal differentiation.

The physiological target genes forSRY/Sry remain unknown, but potential candidates including Sox9, SF-1, DMRT1, GATA-4, Dhh, and testatin, are up-regulated during testicular differentiation 28, 41. Genes SF-1 and WT1play key roles in both sexes in the development of the indifferent gonad. SF-1and WT1, together with SOX9, and GATA4, cooperate to regulate the expression oftarget genes (e. g., AMH, Insl3, and the steroid hydroxylases) that mediate malesexual differentiation.

DAX-1, a negative regulator of the male developmentalpathway, inhibits the activation of critical target genes by SF-1, WT1, SOX9, and GATA4. GATA4 and FOG2 and their physical interaction arerequired for normal gonadal development, WT1 and SF1, which are expressedprior to SRY and necessary for gonad development in both sexes. The tissue distribution of DAX-1 (adrenal cortex, gonads, hypothalamus, andpituitary) is the same as that of another orphan nuclear receptor, steroidogenic factor 1 (SF-1) that is required for development of the adrenalglands and gonads. Dmrt is also one ofthe gene involved in testes differentiation in higher and lower vertebrates, DMRT1 geneencodes a zinc finger–like DNA-binding protein and is expressed very early in asex-specific manner in male gonads of all the classes of vertebrates, regardless of the sex-determining mechanism (chromosomal or environmental).

Inmice, Dmrt1 is expressed in genital ridges of both sexes andthen becomes testis specific at the end of the sex-determining stage. Intestis, Dmrt1 is expressed in germ cells and Sertoli cells 42, 43have recently shown that Dmrt1 is required for testis but notovarian differentiation. Hormones produced by the testis trigger thedevelopmental process that leads to the male phenotypic sexual differentiation 21, independence of gonads and gonadal hormones in normal female birth. There arethree essential hormones secreted by the testes, androgens, MIS, and Insl3. Thesehormones secreted by testes called testicular hormones in male-specificdevelopment of the bipotential reproductive system.

Mullerian-inhibitingsubstance (MIS), also named Mullerian-inhibiting factor (MIF) or anti-Mulerianhormone (AMH), produced by fetalSertoli cells induces

regression of the Mullerian ducts. SexdifferentiationSex is usually defined bythe presence or absence of the sex specific chromosome assin case of mammals. Hermaphroditism is also a common feature of several fishspecies. It was observed that few genes have been linked in the process ofsex determination or differentiation in zebra fish. The genes FushiTarazu factor-1 (FTZ-F1) play crucial role as they wereinvolved in regulating interrenal development thereby steroid biosynthesis, aswell as they also showed expression patterns similar with reproductive tissuedifferentiation and function. It was observed that it can be sex reversed byexposure to estrogens, suggesting that the estrogen levels play a crucial roleduring sex differentiation.

The Cyp19 gene product aromatase usually convertstestosterone into 17 beta-estradiol but when inhibited leads to male to femalesex reversal. FTZ-F1 genes are strongly linked to steroid biosynthesis as wellas regulatory region of Cyp19 contains binding sites for FTZ-F1 genes, furtherlinking FTZ-F1 in this sex differentiation process 44. Since nosex-linked genes have been found fish or lower vertebrates, allelic variantsand dosage effects of autosomal genes, such as the Fushi Tarazu factor-1(FTZ-F1) genes, WT-1, SRY HMG box related gene 9 (Sox9), Anti-Mullerian Hormone (AMH), GATA4 (a zincfinger transcription factor) and double sex-mab 3 related gene (Dmrt1) might beinvolved in determining sex and directing gonad development. The Dax-1 gene hashowever been identified in the Nile Tilapia 18, 45, suggesting that otherfish species may also have Dax-1 homologues that play a role in sexdifferentiation. Several HMG-box containing genes, Sox-genes, have beenidentified in fish 46-48. It was found that HMG-Boxcis

element has been identified in gene promoter of fushi tarazu factor1a (ff1a) 49. Sox9a was also able tobind specifically at this site in vitro (von Hofsten etal., unpublished) indicating that a regulatory connection between Sox9aand ff1a is present.

It was also observed that Sox9 alone does not direct sexdetermination and differentiation in zebra fish. Fish usually lack Mullerianducts, but other AMH functions may be important for gonad development. AMHinhibits the expression of aromatase in developing gonads 50 therefore negativelymodulates the differentiation and function of Leydig cells by down regulatingseveral enzymes involved in the steroidogenic pathway 51. WT1 has been shownto be expressed in the intermediate mesoderm prior to and during renal tissuedifferentiation 52. It is also essential forthe steroidogenic interrenal development together with ff1b 53, 54. WT1 is thereby animportant factor in the early events during development of gonadal primordium. Dmrt1 also play an important role in testis determination in teleosts, sincealteration of aromatase levels during sex differentiation can cause sexreversals 55.

These Dmrt is usuallyregulated by GATA factors. GATA factors processes the binding sites of cyp19gene promoter that indicates its role in regulating aromatase expression 56, 57. SignalTransduction pathwaysSteroid hormone biosynthesis normally occurring insteroidogenic cells is regulated by trophic hormone activation of proteinkinase A (PKA) signaling pathways.

It was observed that this trophic hormonestimulation results in the activation of G proteins which stimulateadenylatecyclase activity that produces

increased intracellular levels of cAMPand PKA in mouse 58, 59. During this signaling, many proteins such ascholesteryl ester hydrolase gets phosphorylated along with transcriptionfactors such as steroidogenic factor 1, GATA-4 and cAMP response-elementbinding protein(CREB)/cAMP response element modulator that activates the genessuch as StAR involved in steroidogenesis. 60, 61. However, there was evidence that regulation ofsteroidogenesis can also modulated by signaling pathways without involvingcAMP.

These include growth factors, steroidogenic inducing protein (SIP), macrophagederived factors, chloride ions and calcium messenger systems 62. Several evidences show that growth factors such asepidermal growth factor (EGF) and insulin dependent growth factor (IGF-1), stimulatesteroid synthesis without altering cAMP levels 63-65. It was observed that EGF and IGF-1 uses theMAPK/ERK pathways for steroid synthesis and StAR expression 65, 66. IGF-1 phosphorylated CREB/activating transcriptionfactor-1 and activator protein-1 family member c Jun/Jun D were also found tobe involved in steroidogenesis. Role of Gonadotropins Gonadotropins are released from the pituitary glandand play an important role in steroidogenesis.

They have shown to activate bothp58 and ERK1/2 MAPKs that result in varying effects on StAR expression andsteroidogenesis in ovarian granulosa cells 67-70. Apart from this it was also observed thatinhibition of p38 decreases both P450arom and estradiol synthesis, and theseevents were tightly correlated with the liver receptor homolog-1 and DAX-11expression demonstrating that p38 targets these transcription factors inregulating

steroidogenesis. Other signaling pathways such as ERK/BMK1, JNK/SAPKalso regulate in steroidogenesis.

Gonadotropinreleasing hormone (GnRH) is widely expressed outside of the classical brain inareas of the olfactory brain, telencephalon, preoptic area and midbrain. GnRH, is best known in vertebrates for its expressionin neurons and its role in stimulating the release of gonadotropins from thepituitary gland. In some earlier studies analysis of the genome confirmedshowing that many teleosts have three forms of GnRH each encoding by separategene. Ovary and testis are major sites of interest because they express bothGnRH and GnRH receptors. It was alsorevealed that peripheral GnRH productionis important in the early development and maturation of the gonads of fish, butare not required at least in large quantities when the fish have reachedmaturity even though the GnRH genes continue to be expressed.

Conclusion: Adrenaldevelopment and gonadal development are two most fundamental biologicalprocesses. Various signalingmechanisms and transcription factors and several pathways are involved atgenomic level in common to adrenal and gonadal development in fishes and lowervertebrates during steroid biosynthesis. Sex determinating factors such as FTZ-F1 genes, Sox9a, GATA4, Dmrt1 and AMH, are involved in the differentiation of gonads. Thestudies also summarized the role of signalling pathway involving ERK1/2, JNK/SAPK, and ERK5 MAPKs in regulation of StAR expression duringsteroidogenesis in different steroidogenic tissues. This emphazise variousgenetic events are involved at the early and late development of the process ofgonadal development in steroidogenic tissues.