Cell - to Species-Level Diversity of Epigenetic Setting for Androgen Receptor Expression in Mammals

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Abstract

During mammalian development, androgen circulates throughout the body and masculinizes several tissues through endocrinological pathways by binding androgen receptor (AR). At the onset of brain masculinization/defeminization, the androgen-AR system functions in a region-specific manner and, even in adulthood, this system affects the transcription of a certain set of genes. The androgen-AR system, together with several coregulators such as histone modifiers, epigenetically regulates many kinds of genes to express the phenotype of a cell according to the cell’s own androgen-sensitivity as well as the dose of androgen to which it is exposed. Long-range DNA-protein interactions via chromatin looping structures also set up epigenetic regulatory mechanisms that affect the androgen responsiveness. Importantly, the androgen-AR system regulates the transcription of AR itself. For such autoregulation, there are a variety of cis-elements within the coding sequences as well as in the regulatory region of AR, including various androgen response elements. We found that some of these cis-elements diverged across species: among them there are several primate-specific regions and rodent-specific regions including a short interspersed element, called B2 SINE, as shown by comparisons between primates and rodents. These data suggest that the gain and/or loss of cis-elements by deletion, insertion and mutation determines the species-specific regulation of AR transcription. Differences in the sequences of AR/Ar regulatory regions may contribute to species-specific transcription regulation in genetic and epigenetic manners. Studies focusing on the biodiversity of the AR regulatory region are important for understanding the diversity of the epigenetic setting determining the responsiveness of cells to androgen.

Keywords: Androgen receptor, Epigenic regulation, Autoregulation, DNA methylation, Pseudogene, Biodiversity

Introduction

SRY is a sex-determining gene on the Y chromosome in eutherians [1]. Certainly, it is SRY which plays the main role in sexual determination of the gonads. During gestation, SRY initiates testis differentiation by activating male-specific transcription factors on autosomes. Although both males and females theoretically have the ability to express androgen receptor (AR) because the responsible gene is located on the X chromosome, SRY directs generation of the testis, which produces androgen and thereby causes the male-biased androgen production. In this way, the androgen-AR system masculinizes tissues other than testis through endocrinological pathways. In mammals, androgen is produced largely in gonadal tissues and plays important roles in sex-dependent development and behavior expression by binding with AR in cell nuclei. For example, testosterone, which is a type of androgen, is primarily secreted by the testes and ovaries, and small amounts are also secreted by the adrenal glands [2]. In both male and female rodents, endocrine disturbance at the fetal and/or postnatal stage irreversibly changes the expression level of genes targeted by androgen-AR [3,4]. Resultant abnormalities of morphology and behavior such as lordosis in males and mounting in females occur after the pubertal stage. This means that genetic differences are not solely responsible for the sex-dependent compositions of the cells in the brain and the nature of many behavior patterns. Rather, circulating androgens are required both for development to fully masculinize the brain structure and for masculine behavior in an epigenetic manner. In fact, the AR expression pattern has diverged in mammals [5]. In this review, we summarize the present understanding of transcription regulation by androgen. Especially, cell- to species-level regulation of AR expression and the diversity thereof through genetic and epigenetic mechanisms will be featured.

Quantitative and Qualitative Effects of Androgen on Transcriptional Regulation

In the testis, androgen functions as a paracrine hormone: it is produced in Leydig cells and acts locally in Sertoli cells in order to support sperm production [6,7]. In addition, fluctuation of the androgen level plays crucial roles in many biological processes in brain and other tissues. For instance, in fetal male rats, the concentration of plasma testosterone dramatically increases up to ~2.2 ng/ml from day 18 of gestation [8]. This rise is a male-specific feature. After day 18, the plasma concentration of testosterone in fetal males decreases steadily to ~0.7 ng/ml on day 22, but on day 23 the level rises again up to ~1.3 ng/ml. This transient upregulation of local testosterone concentration, i.e., the "androgen shower", causes the brain masculinization [9]. Another example is that, during testis maturation, the production of luteinizing hormone (LH) and of follicle-stimulating hormone (FSH), which are differentially but significantly affected by androgen [10], increase at an early pubertal stage, followed by genitalia development and spermatogenesis [11,12]. Through adulthood, testosterone, whose serum concentration is kept at ~4.5 ng/ml, maintains the spermatogenesis in combination with FSH in rats [13]. The AR expression level as well as the local concentration of androgen might...
be, at least in part, involved in shaping the distinct morphological and physiological features in male and females.

In the adrenal gland, androgen functions as an autocrine and/or a paracrine hormone: the zona fasciculata and zona reticularis contain a large number of AR-positive cells. It is known that adrenal androgen is secreted by the zona reticularis [14]. Dehydroepiandrosterone (DHEA), one of the androgens, is the major secretory steroid product of the adrenal gland. In humans, the androgen-AR system in adrenal glands also seems to be affected by endocrine pathways, because, for example, chemical castration (administration of luteinizing hormone releasing hormone agonist) causes an increase in LH and FSH levels, which is followed by an increase in the level of adrenal DHEA [15,16].

The sensitivity of cells in the mammalian brain to androgen varies depending on the expression level of AR. The expression pattern of AR shows a brain-region-specific pattern. AR-containing neurons are widely distributed, with the greatest densities of such cells in the hypothalamus of male and female rodents [17]. The best examples are the medial preoptic area and ventromedial nuclei of the amygdala, the amygdalolimbicopacampal area, and the bed nucleus of the stria terminals [19]. The brain-region-specific expression pattern of AR and localization of AR protein in cells are sex-dependent. In the male human brain, the level of AR protein in the cell nuclei is generally higher in neurons of the lateral mamillary nucleus, the horizontal limb of the diagonal band of Broca, the diagonal band of Broca, paraventricular nucleus, sexually dimorphic nucleus of the preoptic area, suprachiasmatic nucleus, VMN, infundibular nucleus and the medial mamillary nucleus than that in the corresponding regions of the female brain [18]. In most brain areas of humans, there is more cytoplasmic AR protein in male neurons than female neurons, suggesting that this sex-dependent intracellular localization of AR may also have functional significance.

Depending on their androgen sensitivity, AR-expressing cells undergo various processes such as cell migration, cell proliferation and apoptosis in response to similar doses of dihydrotestosterone (DHT). For example, DHT upregulates AR, cyclin A, cyclin D1, and vascular endothelial growth factor gene expression in a dose- and time-dependent manner for the induction of cell proliferation in primary human aortic endothelial cells [19]. In certain prostate cancer cell lines, cell survival and growth are promoted by acetylated and ligand-bound AR [20]. In contrast, AR knockdown by siRNA induces cell growth retardation in a prostate cancer cell line, LNCaP [21]. Androgen can induce apoptosis. Significant decreases in thymic cell lines occur 2–4 h after testosterone cypionate (TC) is administered to castrated male mice [22]. In this change, TC-induced apoptosis plays a role in modulating the size and composition of the thymus. AR also plays indispensable roles in mitogen-activated protein kinase kinase-1-induced apoptosis in prostate cancer cells [23]. In PC-3(AR2) cells with stably transfected AR, DHT functions in the induction of cell growth inhibition and apoptosis [24]. The tumor suppressor BRCA-1 is also involved in DHT-induced cell death through AR transactivation [25,26]. Considering the differential but robust effects of DHT in cells, the epigenetic choice during critical stages, including the timing of androgen-induced brain masculinization, should be important for the differential regulation of AR target genes in cells, and will therefore be further discussed below.

Gradual Effect of Androgen Withdrawal on Cells Through Epigenetic Regulation

Currently, histone modifications and DNA methylation are known as major players engaged in the mechanism of epigenetic setting in mammals. A wide range of species, including unicellular organisms, utilize histone modification for transcriptional regulation [27]. In contrast, DNA methylation is utilized in a more restricted set of species. In fact, the genome-wide DNA methylation level varies depending on the species: there are only trace amounts of methylcytosine in Drosophila melanogaster [28,29], whereas in mammals, DNA methylation mainly occurs at the cytosine in the CpG dinucleotide and is utilized as a primary mechanism for generating the cell- and tissue-dependent gene expression pattern [30–33]. Because mutations in genes associated with DNA methylation cause many defects in neural systems such as Rett syndrome and ICF syndrome [34–36], DNA methylation should play critical roles in the development of the central nervous system. The DNA methylation system in cells displays robustness; for example, induced pluripotent stem cells generated by using factor-based reprogramming of adult murine tissues harbor residual DNA methylation signatures characteristic of their somatic tissue of origin [37]. Long-term exposure of cells to sex steroid hormones may induce sex-dependent DNA methylation patterns. Indeed, androgen-AR containing complexes consist of many kinds of proteins such as histone modification enzymes, which affect the epigenetic status of target gene promoter regions [38–40]. Moreover, testosterone propionate (TP) or DHT supplementation influences the status of histone modification, nucleosome occupancy and DNA methylation, as discussed in detail later [41–43].

Because of the involvement or lack of involvement of the estrogen-ER system in the brain masculinization in rodents and primates, respectively [44], we do not yet know if the androgen-AR system is commonly used for epigenetic setting of the brain across species. Indeed, androgen priming of the cells determines the sensitivity of these cells to androgen itself in later stages of life. For example, in adulthood, the concentration of circulating androgen can influence the size of certain brain regions in mammals [45]. In the posterodorsal medial amygdala, neuronal soma size is greater in male rats than in females, and this sexual dimorphism is maintained most probably by circulating androgen. One month after surgical castration of adult males, these brain regions shrank to a size indistinguishable from that in females without changing the cell number, whereas TP treatment of adult females enlarged these regions to sizes similar to those in males [45]. This response to TP in the adult brain can be regarded as an epigenetic phenomenon because the cells changed their phenotypes without proliferation. If androgen sets a specific epigenetic status, it appears to be difficult to alter it later. This hypothesis is supported by the cell behavior upon androgen withdrawal. Long-term deprivation of DHT or TP from the culture medium of prostate cancer cells can convert some cells to clones that are completely independent of androgen for their cell survival [46,47]. Even in these androgen-insensitive cells, CASP8, which is normally upregulated by androgen-AR, is still expressed irrespective of androgen. Similarly, TMS1, which is downregulated by androgen-AR, still keeps its repressed status. These transcription states can be altered by treatment with 5-aza-2’-deoxycytidine, a drug that alters the DNA methylation pattern [48]. In fact, differences in genome-wide DNA methylation and gene expression profiles have been observed between AR-positive and AR-negative cancer cells. Taken together, these findings suggest that androgen signal primarily maintains the status of the cell physiology, and later can be replaced.
by a more stable mark by which the cell “memorizes” the androgen-dependent gene expression pattern even in the absence of any steroid hormones.

**Effect of Androgen-bound AR on Epigenetic Regulation**

In the cell nucleus, AR dimerizes and binds to a specific sequence known as the androgen response element (ARE). Occupancy of ARE by the androgen receptor complex can lead to the acute upregulation of the physically associated gene [49,50]. Glucocorticoid, mineralocorticoid, and progesterone receptors and AR constitute a group of proteins that form homodimers capable of recognizing three-nucleotide-spaced inverted repeats (IR3) nestled between two 5'-TGTTCT-3' [51,52]. However, this consensus sequence sometimes does not effectively predict AR-binding regions in androgen target genes [53-55]. Therefore, androgen-responsive sequences were further collected experimentally, and it was found that the 3' half site of ARE, but not the 5' half, was commonly utilized for recognition by liganded AR [56]. In other words, androgen-AR can recognize diverse sequences together with a subset of epigenetic regulators such as methyl-binding proteins and histone modifiers, leading to the differential gene expression depending on the cell [38].

Regarding histone modifiers, CBP/p300 and LSD1, which possess histone acetyltransferase and demethylase activities, respectively, [57,58] seem to function as coactivators together with AR. In vitro studies indicate that androgen-AR is present together with CBP, acetylated histones, and RNA polymerase, on the promoters of active AR- as well as ER-target genes, suggesting that histone acetylation might play an important role together with AR [59]. In a different context, AR mediates gene repression [60]. A direct protein-protein interaction among 5'TG3' interacting factor (TGIF), AR and a transcription corepressor, Sin3A, recruits HDAC1 to repress the target gene. In this context, differential utilization of partner proteins of AR depending on the cell may underlie the sequence-specific epigenetic setting. The expression of partner proteins of AR is sex-dependent. For example, the level of CBP in the rat hypothalamus is higher in males by antisense oligonucleotides causes behavioral feminization of males [61]. Regarding DNA methylation, AR could affect the formation of sex-dependent DNA methylation patterns in response to androgen. In the cerebral cortex of mice, amyloid precursor protein (App) promoter methylation is higher, and App mRNA expression is lower in females than in males. Treatment with TP decreased the DNA methylation level of the App promoter region [62]. Thus, both DNA methylation and histone modification are involved in epigenetic setting of androgen-AR targets. It should be noted that AR itself is targeted for androgen-dependent DNA methylation regulation in a cell-type-specific manner. The impact of the auto-regulation by androgen-AR is described in detail below.

In addition to the epigenetic regulators in AR-expressing cells, the tertiary structure made by long-range DNA-protein interactions may also determine the androgen responsiveness. Biochemical and genetic studies have revealed that cooperation between the enhancer and the promoter in a prostate-specific antigen gene results in maximal androgen responsiveness [63-70]. Using the 3C assay, which makes it possible to identify long-range DNA-protein interactions, it was found that both androgen and FOX1A, a major FOX family member [71-73], are required for chromatin looping in LNCaP cells [74]. In this system, Med12, one of the components of the androgen-AR complex, mediates enhancer-promoter looping [75]. Moreover, a similar phenomenon can also be seen in the promoter region of genes targeted by estrogen-bound ERα, [76], raising the possibility that looping-structures could perform local transcriptional regulation [77]. In accord with the idea of ERα-mediated epigenetic setting, the androgen-AR system thus appears to be involved in differential chromatin looping that may somehow be fixed for long-range regulation of gene expression (Figure 1). Genes within such a looping-structure showed coordinated upregulation of their transcription by supplementation of steroid hormones. The regions close to the AR-bound regions showed a tendency to rapidly constitute active chromatin structures reminiscent of the active gene transcription in response to androgen. A specific gene cluster might be organized to restrict the epigenetic effects of the androgen-AR complex within a loop to strengthen the coordination of the transcription, and this structure would isolate this effect to prevent leaky transcription of genes located outside of the loop. Such differential AR-triggered epigenetic setting depending on the cell type may account for the tissue- and species-dependent differences of cell fates resulting from various degrees of responsiveness to androgen.

**Cell-type-dependent Autoregulation of AR**

Figure 2 summarizes the cis-elements that potentially act to generate commonality and inter-cell diversity of the AR expression pattern in human cells. Notably, transcription factors that potentially bind to the cis-elements listed in Figure 2 frequently show cell-type-dependent expression patterns within a species. The promoter for AR exhibits a GC-rich character and the absence of a TATA box [78]. This core promoter contains an Sp1 binding site (-46/-37) and helix-loop-helix (HLH) protein binding site (-19/-14) [79]. Immediately upstream, there is a long homopurine stretch (-117/-60) which is important for the AR promoter activity and conserved in rodents and primates. Multiple weak Sp1 binding sites in this homopurine region increase the supply
of this transcription factor to the minimal promoter to facilitate the assembly of the transcription initiation complex [80]. In the 5′ flanking sequence, there also proved to be binding of NFκB [81,82], B-myb [83], cAMP response element binding protein (CREB) [84], NF1 [85] and HLH protein [79]. In addition, the region from the transcription start site (TSS) to -2.3 kb contains predicted binding sites for NF1, API and CREB, and also retinoic acid response element (RARE), interleukin-6 responsive element (IL6-RE) and 2,3,7,8-tetrachloro-dibenzo-p-dioxin responsive element (TCDD-RE) sequences [86]. This huge variety of cis-elements enables complex transcriptional regulation via association with trans-acting factors.

Actually, AR itself is a factor involved in the regulation of AR expression. The androgen-AR system could affect AR promoter activity through AR binding to the multiple AREs [86-88]. Reporter assays with the proximal 5′-flanking region of the TSS reproduced this autoinduction by androgen [89]. AREs are also located in exons 4 and 5 of AR, spanning -350 bp of the AP-1 DNA binding motif and -6.5 kb of the genomic DNA [90,91]. This 350-bp region also contains a Myc consensus site where interaction of Myc with Max, a factor dimerizing with Myc and involved in cell fates [92], occurs for androgen regulation [91]. These kinds of autoinduction via the interaction of androgen-AR with multiple AREs occurs in a cell-specific manner, reflecting the cell’s sensitivity to androgen. Therefore, it is plausible that the autoregulatory loop for setting androgen-AR sensitivity in cells shares the common circuit regulating the endocrine environment. This hypothesis is supported by the fact that, for example, the 350-bp region is androgen regulated in U2OS and PC3 cells, in which androgen methyl trenolone (R1881) or DHT can induce AR expression [89,93], but not in LNCaP cells, in which R1881 downregulates AR expression [94,96]. Thus, epigenetic regulation by forming specific DNA methylation patterns and tertiary structures may underlie the characteristics of AR-expressing cells. Indeed, an ARE located in exon 4, which is >150 kb distant from the TSS, could participate in the transcriptional regulation of AR, suggesting that the looping structures help to keep AREs close to the TSS. Since DNA methylation plays a critical role in the transcription of AR [97-99], it would be interesting to clarify the molecular mechanism governing the epigenetic setting of AR expression by androgen-AR together with factors regulating DNA methylation in a cell-specific manner.

Possible Contribution of Species-specific cis-elements for AR to its Transcription

Studies focusing on the homology of proximal and distal regulatory regions of AR/Ar between primates and rodents will lead to better understanding of the species-specific regulation. Differences in the transcriptional regulation of AR/Ar between primates and rodents may be responsible for functional divergence of the responses to endocrinological and environmental cues. Actually, the mechanism of brain masculinization/demasculinization involving sex-steroid hormones (androgen and estrogen) is different between primates and rodents. Androgens are required to fully masculinize the brain structure during development and the behavior in adulthood. In rodents, estradiol is made by the aromatization of testosterone and interacts with ER for brain masculinization [100]. In contrast, there is little evidence that aromatized metabolites of androgen play this role in primates, including humans [44,101]. There are other examples in which androgens themselves also masculinize the brain through their interaction with AR in mice and rats [44,102,103]. The brain-region-specificity of AR expression is also different between primates and rodents [5], perhaps due to species-dependent DNA elements and/or their epigenetic modifications. Considering that the androgen-AR system triggers brain masculinization in both rodents and primates, the responsiveness of AR expression to estrogen-ER may be different between these orders. This hypothesis is supported by the fact that transcriptional regulation of AR/Ar by estrogen differs depending on the species [77]. However, ER-binding sites have not been identified in the AR/Ar regulatory regions. Therefore, comparing various features of the species-dependent differences of the AR regulatory
region might be important for understanding the dependence on or independence of estrogen of various biological phenomena, including brain masculinization, in different mammals.

The proximal regulatory region of AR is highly homologous (~95%) in the human and macaque genomes. In contrast, the corresponding regions have diversified between the mouse and rat genomes. From the dot plot analysis of the Ar locus in mice and rats, we identified a mouse-specific region (-1806 to -1603) in the proximal region of mouse Ar (Figure 3A). Interestingly, homology search using the BLAST algorithm indicates that this region contains a short interspersed element (SINE), called B2. In the mammalian genome, there are many SINEs, which are propagated by retrotransposition. The B2 class of the SINE family constitutes approximately 0.7% of total mouse genomic DNA [104]. The B2 SINE family has the potential to distribute a functional cis-element throughout the genome [105]. For example, the B2 SINE in the promoter region of Lama3 is bound by the transcription factor USF to regulate the gene transcription. The transcription occurring at B2 SINE itself can also shape a given tissue [106]. The B2 SINE

Figure 3: Divergence of the 5'-flanking sequences of AR/Ar. A. Dot plot of the mouse Ar and human AR relative to the rat Ar locus. In this plot, sequences with high similarity were plotted in a 50-bp window. B. Sequence comparison of 5' region (-15 kb to +2 kb) and CDS of AR/Ar across species using the BLASTZ algorithm. Data were visualized with the software GenomeJACK (http://www.mss.co.jp/businessfield/bioinformatics/solution/products/genomejack/index.html). Each colored bar in the row for the indicated species represents a particular chromosome where the sequence best matched to the human genomic sequence in the red bar is located. Asterisks denote half-sites of consensus estrogen responsive element (5'-GGTCAnnnTGACC-3': [114]).
transcribed in murine growth hormone-expressing cells regulates the gene expression in a cell-specific manner. At this gene locus, the transcription of B2 SINE functions as a boundary to block the influence of repressive chromatin modifications. Therefore, one can easily speculate that the B2 SINE insertion in the 5’-flanking sequence of Ar in mice generates the species-dependent Ar regulation by altering the chromatin structure for epigenetic regulation. In addition, the rat proximal regulatory region contains three small specific sequences (~2639 to -2642, -1150 to -1133 and -314 to -302; arrowheads shown in Figure 3A). Unlike the mouse-specific regions in Ar, these rat-specific regions do not contain any consensus sequences, and therefore it will be necessary to experimentally identify the factors that mediate their species-dependent regulation of Ar. In the same way, from the dot plot analysis of the AR/Ar locus in humans and rats, we identified a rat-specific region (~2566 to -2186) in the proximal region (Figure 3A). This element might be inserted into and disrupt a CRE, which may be involved in an autoregulatory loop of the androgen-AR system. Exon 1 of human AR contains CAG repeats (+1287 to +1388), which are frequently targeted to be expanded up to ~36 repeats even without any phenotypic abnormality. In disease conditions, the repeats increase to 38~65. The CAG repeat expansion is linked with diseases such as prostate cancer, breast cancer and SBMA [107-109]. The average number of CAG trinucleotide is different between species: 29 in humans, 19 in rats, and 12 in mice. In humans, there is an inverse relationship between CAG repeat length and the expression level of AR [110]. Therefore, the species difference of the CAG repeat number might also influence the divergence of the transcriptional regulation of AR/Ar.

One example in which the species difference of a cis-element contributes to morphology is a human-specific deletion located ~200 kb downstream of the transcription termination site of human AR and spanning ~60 kb. This deletion removes a sensory vibrissae and penile spine enhancer from human AR, a molecular change correlated with anatomical loss of androgen-dependent sensory vibrissae and penile spines in the human lineage [111]. Ablation of spines decreases tactile sensitivity and increases the duration of intromission [112]. McLean CY, et al. [111] could not exclude the possibility that loss of the AR enhancer occurred because of relaxed selection following other genetic changes that led to anatomical differences in the human lineage. However, based on the previously established role of AR in vibrissae and penile spine development, the authors hypothesize that deletions of tissue-specific enhancers in AR have contributed to both loss and expansion of particular tissues during human evolution. It is still possible that species differences in the AR/Ar regulatory region are the result of genetic drift.

These inter-species comparisons of the proximal regulatory sequences allow us to propose a hypothetical model for the acquisition/mutation of the pseudogene in the regulatory region of AR/Ar during evolution. We compared the proximal regulatory sequences of AR/Ar among humans, chimpanzees, gorillas, macaques, mice, rats, bovines, pigs, dogs and chickens (Figure 3B). In primates, a pseudogene for a poly(A) binding protein, nuclear 1, named PABPN1, is located there, whereas the corresponding sequence is not found in mice. In rats, bovines, pigs and dogs, there are sequences reminiscent of PABPN1, but the 5’ end of this sequence seems to have diverged from that in humans, suggesting the occurrence of retrogene insertion followed by accumulation of mutations in the 5’ flanking sequence of AR/Ar. Nonetheless, an Ensembl transcript (Accession number: ENST00000449099) is well aligned with this divergent DNA element, which may suggest the utilization of this sequence for species-dependent RNA generation at the AR/Ar locus.

Whereas mutations in the coding sequence (CDS) may easily cause lethal phenotypes by causing amino acid substitutions, mutations in the regulatory regions might diversify the transcription factor binding, and thereby change the expression level without causing protein structure alterations [113]. Regarding this possibility, we also compared the CDS of AR/Ar among humans, chimpanzees, gorillas, macaques, mice, rats, bovines, pigs, dogs and chickens, and found that it is strongly conserved across mammals but not across vertebrates (Figure 3B). Figure 3C shows a summary of the diversity of the AR/Ar proximal regulatory regions. Deletion, insertions and the accumulation of mutations may have been positively fixed to confer the observed divergent and species-specific aspects of transcription regulation. The commonality and diversity of the cis-element in the proximal region of mammalian AR/Ar described above will be helpful for understanding the species-dependent epigenetic setting for AR/Ar transcription.

Concluding Remarks

AR can be involved in the epigenetic regulation of a subset of genes, including AR. Diversified as well as conserved sequences in the 5’-flanking region of AR seem to be utilized for the epigenetic setting. Here, we emphasize the importance of not only the common aspects of androgen for cell physiology, but also species-specific phenomena, including sexual behavior, that are primarily driven by the genetic differences in the regulatory region of AR affecting its epigenetic setting. These cis-elements appear to be utilized as “epigenetic switches” participating in cell-, tissue-, and/or species-specific regulation with the ability to turn on and/or off the transcription of AR through epigenetic regulation by DNA methylation and histone modifications.
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