Maternal regulation of estrogen receptor α methylation
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Advances in molecular biology have provided tools for studying the epigenetic factors that modulate gene expression. DNA methylation is an epigenetic modification that can have sustained effects on transcription and is associated with long-term gene silencing. In this review, we focus on the regulation of estrogen receptor alpha (ERα) expression by hormonal and environmental cues, the consequences of these cues for female maternal and sexual behavior, and recent studies that explore the role of DNA methylation in mediating these developmental effects, with particular focus on the mediating role of maternal care. The methylation status of ERα has implications for reproductive behavior, cancer susceptibility, and recovery from ischemic injury, suggesting an epigenetic basis for risk and resilience across the life span.

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Introduction
The coordination of endocrine signals is essential to successful reproduction, particularly among mammals in which there is extensive prenatal and postnatal interaction between mothers and infants. During late gestation, circulating levels of estrogen increase and are essential for the upregulation of peptide receptors involved in parturition, lactation, and maternal behavior. Thus, sensitivity to estrogen is crucial to the change in behavior that promotes growth and survival of offspring through maternal investment. At a cellular and molecular level, estrogen is known to act through two distinct pathways: (1) through intracellular signaling following activation of membrane bound estrogen receptors and (2) through more classical genomic routes in which estrogen binds to nuclear estrogen receptors leading to transcriptional activation. Consequently, levels of estrogen receptor will determine the sensitivity to this hormone and ultimately regulate the efficiency of estrogen-mediated signaling and the biological and behavioral outcomes associated with estrogen. Recent evidence suggests that there are pervasive effects of the environment on the expression of estrogen receptors with implications for health and reproductive behavior. In particular, the alpha isoform of the estrogen receptor (ERα) has been found to be dynamically altered through epigenetic modification in response to physiological and behavioral cues. In this review, we will discuss emerging evidence for the role of environmental signals in regulating ERα, the role of DNA methylation in mediating these effects, and the implications of these interactions between gene and environment on reproduction within and across generations.

ERα and female reproductive behavior
Estrogen receptors belong to the nuclear hormone receptor family and dimerize in response to ligand binding to form a complex that promotes transcriptional activation of genes containing estrogen response elements (EREs). Though there are also non-genomic pathways of estrogen action involving membrane bound receptor activation and intracellular signaling with significant implications for physiology and behavior [1,2], the induction of transcription through activation of the two nuclear estrogen receptor isoforms, ERα and ERβ, are considered the classic route of estrogen effects [3]. Though both ER isoforms are expressed within the brain and have similar DNA binding domains [3,4], ERα and ERβ differ in ligand affinity and in the conformational changes that occur as a function of ligand binding [5]. Consequently, ERα has a greater affinity for estrogen, and activation of this receptor isoform is associated with comparatively higher levels of transcriptional activity.

Pharmacological and genetic manipulations have been used to illustrate the role of ERα in the reproductive behavior of both male and female rodents. High levels of ERα expression are found in the hypothalamus, with particularly elevated expression within the medial preoptic area (MPOA), as well as the amygdala and ventral medial hypothalamus (VMH) [4]. The MPOA is crucial for male sexual behavior [6] and female maternal behavior [7], whereas the VMH has been found to regulate female sexual receptivity [8]. Site-specific administration of the estrogen receptor antagonist 4-hydroxytamoxifen in the MPOA disrupts the onset of maternal responsivity among post-parturient females, whereas administration of this antiestrogen into the VMH blocks the occurrence of the postpartum estrus [9]. ERα continues to influence
maternal behavior during the postpartum period as indicated by c-fos activation in ERα positive cells in lactating female rats [10]. Targeted disruption of the ERα gene has been found to dramatically reduce the occurrence of lordosis and lead to increased rates of rejection of male attempts to initiate copulation [11]. These ERα knockout females also show elevated levels of infanticide and reduced motivation to retrieve pups indicating a broad spectrum of reproductive impairment. These behavioral deficits may be the consequence of abnormal development and regulation of oxytocin [12] and dopaminergic neuron signaling [13*]. Mutation of ERα results in an elimination of estrogen-mediated upregulation of oxytocin receptor binding in several brain regions [12], and recent evidence suggests that striatal tyrosine hydroxylase levels are decreased in ERα-KO mice [13*], which may account for the physiological, motivational, and motor aspects of reproductive impairment in these females.

Early environmental regulation of ERα
One strategy for understanding the role of the environment in regulating ERα is to examine the consequences for ERα expression of developmental exposure to hormones, endocrine disruptors, and peptides. Sexual dimorphism in ERα expression, with reduction in hypothalamic ERα in males compared with females, emerges developmentally [14] and is sustained into adulthood, suggesting the organizational effects of circulating estrogens. Early treatments with elevated levels of this hormone have been found to decrease levels of ERα in the female brain and eliminate sex differences in ERα expression [15]. The widespread use of xenoestrogens such as bisphenol A (BPA) in the manufacture of household plastics has led to more thorough examination of the neuroendocrine and behavioral consequences of long-term exposure to the effects of synthetic estrogens. Neonatal treatment with high levels of BPA initially induces an increase in ERα with subsequent decreases in ERα expression within the MPOA [16**]. The reproductive consequences of BPA-induced changes to hypothalamic estrogen receptors early in development include reduced sexual differentiation and significant reductions in the duration of maternal licking/grooming (LG) and frequency of nursing of pups during the postpartum period in adult females who were BPA-exposed as neonates [17,18]. Recent in vivo and in vitro studies have also shown that peripheral administration of oxytocin to female pups during the postnatal period can increase ERα in the VMH, whereas administration of a selective oxytocin receptor antagonist can decrease ERα immunoreactivity in the MPOA [19,20*]. These developmental effects on ERα are observed within the pre-weaning period and are sustained in adulthood. This may account for long-term oxytocin treatment effects including observed increases in adult sexual and social behavior [19,21].

Though direct targeting of ERα through pharmacological manipulation of the neuroendocrine system certainly has profound effects on behavior, similar regulatory influences can be achieved through modification of the early social environment. In rodents, natural variations in maternal care during the postpartum period are associated with long-term effects on offspring gene expression, physiology, and behavior [22]. Comparison of the offspring of rat dams who engage in high vs. low levels of maternal LG indicates that exposure to low levels of this form of maternal care are associated with decreased hippocampal glucocorticoid receptor (GR) expression, increased hypothalamic–pituitary–adrenal response to stress and reduced exploration in a novel environment [23]. Female offspring of low LG dams display high levels of sexual receptivity [24,25], yet engage in low levels of maternal LG toward their own offspring [26,27]. Individual differences in maternal LG are associated with variation in central oxytocin receptor density, and ICV infusion of a selective oxytocin receptor antagonist decreases frequency of LG among high LG dams [28]. Female offspring of low LG dams likewise have reduced central oxytocin receptor density and display reduced sensitivity to estrogen-induced upregulation of neural activation and oxytocin receptor density within the hypothalamus [28–30]. This differential sensitivity is similar to what is observed among ERα KO females [12], and analysis of ERα expression in the MPOA as a function of postnatal maternal care confirms that the offsprings of low LG dams have reduced expression of this receptor isoform [29]. Cross-fostering of offspring from high LG to low LG dams or from low LG to high LG dams indicates that this difference in gene expression is associated with the quality of the postnatal environment [31**]. Similar long-term effects on maternal behavior and ERα mRNA expression in the MPOA have been demonstrated in rat offspring who were cross-fostered between dams who were induced to be high or low LG as a consequence of exposure to a predator odor [32*]. Conversely, ERα mRNA in the anteroventral paraventricular nucleus of the hypothalamus is elevated among the female offspring of low LG dams and these females are more sensitive to estrogen induced ERα activation within this region [33**]. Thus, there are multiple hormonal and behavioral cues occurring early in development that exert site-specific regulatory influence on ERα with consequence for multiple aspects of reproduction.

Epigenetic regulation of ERα through DNA methylation
The prolonged alterations in ERα levels that have been observed in response to early life experience suggests stable regulation of gene expression through epigenetic mechanisms. There are many modifications to chromatin structure that can alter transcriptional activity of the genome [34,35]. However, the most stable of these modifications is DNA methylation, in which a methyl group is attached by DNA methyltransferases to cytosine
nucleotides within the DNA sequence. DNA methylation within the gene promoter generally prevents binding of transcription factors and RNA polymerase and is associated with gene silencing [36]. Methylation patterns are stable and heritable, providing a pathway through which cellular differentiation can occur. Despite this stability, there is recent evidence for the dynamic regulation of gene promoter methylation in response to environmental condition, particularly those experiences occurring early in development. In the case of ERα, the differential expression of this receptor in response to variation in maternal care received in infancy has been found to be associated with methylation patterns within the ERα promoter. Comparison of the adult female offspring of low vs. high LG dams indicates elevated levels of ERα methylation at several of the CpG sites within the 1b promoter region in tissue taken from the MPOA of offspring of low LG dams [31**]. Consequently, there is less binding of transcription factors, such as Stat5b (signal transducer and activator of transcription 5b) to the ERα promoter [31**]. Though the cellular/molecular pathway through which maternal care alters ERα methylation has yet to be elucidated, one potential route is through maternal upregulation of transcription factors in the neonatal hypothalamus that promotes ERα transcriptional activity and reduce the likelihood of epigenetic silencing. There is evidence for this activity/transcription factor-dependent pathway in the maternal regulation of GR expression, implicating serotonergic pathways and maternal upregulation of NGFI-A (nerve growth factor-inducible protein-A) [37,38**]; however, this level of analysis has not yet been applied in the context of ERα. These long-term effects could also be indirectly mediated through LG stimulation of estrogen and oxytocin levels in the neonate that has been shown to regulate ERα expression in adulthood [15,20**]. Future studies will focus on the possible routes through which these early behavioral and physiological events lead to modification of the epigenome.

The plasticity in ERα expression that can be achieved through DNA methylation has also been investigated in the context of cancer treatment and recovery following ischemic injury. The dysregulation of cell cycle that is characteristic of rapidly dividing cancer cells is associated with global hypomethylation and site-specific hypermethylation, particularly of tumor repressor factors [35,39]. Elevated levels of DNA methylation of the ERα promoter are found in breast cancer cells, leading to reduced ERα expression and a decreased efficiency of tamoxifen treatment [40], which works through the blocking of ERα. Advances in pharmacological targeting of the epigenome have lead to the development of several drugs that alter DNA methylation levels primarily through promotion of histone acetylation. Administration of histone deacetylase inhibitors, which increase histone acetylation and thereby decrease DNA methylation, in the treatment of cancer provides a novel approach to improving prognosis and in the case of ERα has been found to increase estrogen sensitivity and the efficacy of tamoxifen treatment [41,42]. The origins of ERα expression and methylation abnormalities observed in cancer cells are yet unclear; however, there is recent interest in the potential role of environmental risk factors such as developmental exposure to xenoestrogens that alters DNA methylation and is associated with increased cancer risk [43]. Interestingly, a recent report indicates that the epigenetic abnormalities associated with in utero BPA exposure can be reversed through maternal dietary supplementation with genistein, folic acid, choline, and betaine [44*], which serve as methyl donors within the DNA methylation process [45]. Though these studies are compelling, the site specificity, gene specificity, and developmental timing of these global treatments must be considered in evaluating the consequence of this therapeutic approach.

Changes in the expression of ERα occur across development and within the reproductive cycle. Though ERα levels are elevated in the hypothalamus in both early development and in adulthood, in the cortex there is a significant decrease in ERα in the adult brain [46]. However, ischemic injury results in a rapid increase in cortical ERα in female rodents and may serve to enhance neuroprotection following injury [47]. Estrogen has previously been shown to prevent cortical damage following an ischemic episode [48] and using ERα-KO mice, this effect has been shown to be ERα dependent [49]. Recent evidence suggests that DNA methylation of the ERα promoter is decreased in rodent cortical tissue of females but not males following ischemic injury [50**]. Thus, pre-ischemic and post-ischemic factors that modulate the DNA methylation pathway and the regulation of ERα may determine functional recovery following injury.

Conclusion

The expression of ERα has functional implications for reproductive behavior and health and can be regulated through multiple hormonal and environmental pathways occurring developmentally and in adulthood (Figure 1). Recent evidence suggests that variation in DNA methylation of the ERα promoter can be induced by the quality of the early maternal environment with long-term consequences for the maternal behavior of female offspring. The behavioral transmission of these epigenetic maternal effects from mother to offspring [26,31**] suggests that any environmental condition that can alter ERα expression within the hypothalamus may have implications for the reproductive behavior of subsequent generations. Thus, pharmacological manipulations that target DNA methylation globally and have been shown to modify ERα gene expression could induce transgenerational effects on health and behavior. Our current understanding of the epigenetic regulation
of gene expression and the implications of this regulation for individual differences in physiology and behavior has advanced rapidly through use of molecular and cellular approaches. In future, these studies may provide further insight into the biological basis of the interaction between genes and environment and the developmental origins of long-term reproductive outcomes.

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest


Using ERalpha knockout mice, the authors demonstrate the influence of this receptor for the development of dopaminergic systems in males and females that may have implications for the deficits in social and cognitive functioning previously observed in ERalpha null mice.


Examines the developmental effects of BPA exposure on ERalpha expression and promoter methylation. Though there is a significant effect on hypothalamic ERalpha gene expression, the authors do not find an association with DNA methylation.


Using a model in which monogamous prairie voles are treated developmentally with oxytocin or an oxytocin receptor antagonist, the authors demonstrate the positive association between oxytocin levels in infancy and adult ERalpha expression.


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