

Epigenetics, Evolution, Endocrine Disruption, Health, and Disease

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Endocrine-disrupting chemicals (EDCs) in the environment have been linked to human health and disease. This is particularly evident in compounds that mimic the effects of estrogens. Exposure to EDCs early in life can increase risk levels of compromised physical and mental health. Epigenetic mechanisms have been implicated in this process. Transgenerational consequences of EDC exposure is also discussed in both

a proximate (mechanism) and ultimate (evolution) context as well as recent work suggesting how such transmission might become incorporated into the genome and subject to selection. We suggest a perspective for exploring and ultimately coming to understand diseases that may have environmental or endocrine origins. (Endocrinology 147: S4–S10, 2006)

“Genetics proposes, epigenetics disposes.”
—Medawar and Medawar (1)

ENDOCRINE-DISRUPTING CHEMICALS (EDCs) are compounds in the external environment that mimic or block endogenous hormones. In other words, they behave as biological signals and, as such, can be misinterpreted by the organism. To date, the vast majority of known EDCs are those that activate the parts of the endocrine system associated with the steroid/retinoid/thyroid super-family of receptors, most often receptors related to the hormone estrogen that are found in all vertebrates. Also, many other species, such as crustaceans and mollusks, respond to estrogen, and, surprisingly, plants, corals, and at least one fungus make estrogen-like molecules. Environmental contaminants such as the *ortho*, *para* isomer of dichlorodiphenyltrichloroethane (DDT), some hydroxylated polychlorinated biphenyls (PCBs), the plasticizer *bis*-phenol A, and some detergents such as *p*-nonylphenol, can exhibit estrogenic activity (2).

In this article, we will discuss some aspects of evolution that may help to explain why so many EDCs produce a signal that is interpreted by vertebrate systems as being estrogen. We will also explore another question: can endocrine disruptors actually participate in the evolutionary process? And, finally, we will use information recently uncovered, regarding mechanisms of endocrine and environmental signaling, to explore the role of the environment in health and disease.

EDCs act on individuals. Exposure can be during a very

restricted period or throughout the individual's lifetime. To understand what effect they might have, the individual's social and biological environment, namely its ecology, must be considered. This ecology includes interactions between and within species; for example, symbiotic signaling, predatory-prey interactions, males and females during mating, mother-embryo interactions, and, in mammals, even embryo-embryo interactions. Environmental signal disruption principles also apply to far less mobile species (*e.g.* plants, corals, crustaceans, and mollusks).

To understand the process by which these chemicals work we must look to the gene. Like an organism, a gene is not expressed in isolation but rather in the context of other genes and their products, cells, and tissues in a temporal/spatial dimension (3). This might be thought of as the ecology of gene expression. It is well known that EDCs, and very likely endogenous hormones, can act on a gene's developmental mechanisms, altering phenotype expression. We are now seeing that the mechanism of these phenotypic changes is probably epigenetic; in other words, they cause mitotically heritable changes in gene function without changing the DNA sequence, *i.e.* without mutation. In fact, EDCs do not act on genes alone but on developmental mechanisms that integrate genetic and epigenetic interactions, resulting in the phenotype.

One mechanism for inducing epigenetic change is through DNA methylation, a process by which methyl groups are added to the base cytosine in DNA, usually suppressing expression of the gene. In most studies, increased DNA methylation is associated with gene silencing and decreased methylation is associated with gene activation. Currently, a handful of papers have implicated EDCs in epigenetic programming and DNA methylation (4–7). These changes, when they occur during certain stages of development, are permanent and can be inherited by offspring (6, 8, 9). This inevitably leads us to consider whether such epigenetic changes can be subject to selection.

First Published Online May 11, 2006

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Abbreviations: DES, Diethylstilbestrol; EDC, endocrine-disrupting chemical; ER, estrogen receptor; GR, glucocorticoid receptor; PTSD, posttraumatic stress disorder; TCDD, 2,3,7,8-tetrachlorodibenodioxin.

Endocrinology is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

Epigenetics

Before the 1940s, the gene as the unit of heritable material was a theoretical concept without a physical identity. In 1942, C. H. Waddington (10) reintroduced the term epigenetics from classical embryology, ascribing to it the manifold ways that gene action and expression give rise to the phenotype (11). In developmental genetics today, however, the definition has become far more narrow and specific, referring principally to DNA-related mechanisms of inheritance such as methylation and chromatin remodeling resulting in the monoallelic silencing of genes in early mammalian development. This relatively recent area of research focuses on processes such as DNA methylation and histone modifications that are heritable in the short term but do not change the DNA or create mutations. Because manipulation of methylation patterns is often lethal, or at the least results in maladaptive traits or monsters, this method of research illuminates normal development by creating abnormalities or anomalies. Furthermore, although mechanisms such as DNA methylation are clearly involved in genomic imprinting, the signal for the imprint is not yet known.

Might natural hormones or EDCs be one of the signals? Hormones are known to epigenetically imprint genes in non-mammalian vertebrates. Work on epigenetic memory with the vitellogenin gene in frogs (12) shows that hormonal treatments early in life alter the response of hormonally regulated genes to the same or different hormones later in life. The first hormonal experience epigenetically alters the set point for the later hormone response. This process can be determined, in frogs, by methylation (13). The term epigenetic imprinting has also been used to describe a process in which estrogens in development cause persistent alterations in gene expression and reprogram cell fate (5, 14, 15). It has also been used to describe gene silencing observed in the carcinogenic process as well.

Epigenetic imprinting by EDCs or other hormones represents one potential mechanism for Waddington's concept of genetic assimilation, a direct outgrowth of his research in epigenetics. Based in part on his work with *Drosophila*, Waddington (10, 16) speculated that environmentally induced changes in phenotype could become incorporated into the genome as evidenced by the persistence of the phenotype even after the original selection pressure is relaxed. It is in this manner that selection might act on developmental pathways leading to adaptive change in the genome in conjunction with genetic mutation. Evidence has emerged in recent years that epigenetically mediated changes in phenotype can be stable over many generations. Indeed, in the annually reproducing plant *Linaria vulgaris*, an epigenetically determined variant has been maintained for over 250 yr (17). Furthermore, Ruden and colleagues (18) have documented epigenetic change that can be assimilated in *Drosophila*. As they point out, gene-associated tandem repeat expansions and contractions as well as retrotransposon mobilizations are epigenetically up-regulated during times of environmental and hormonal stress. Because such events can occur at rates up to 100,000 times higher than point mutations, this class of mutation potentially has a much greater effect on morphological evolution than do point mutations (19). This has led

Ruden *et al.* (20) to suggest that the regulation of the CpG methylation status of repetitive sequences in germ cells could increase the rate of morphological variation and thereby the rate of morphological evolution.

Likewise, Holliday (21, 22) has proposed that teratogens and, indeed, hormones could work through patterns of DNA methylation in the embryo, which may lead to developmental alteration in the offspring with persistent changes in the germ line. With regard to exogenous compounds altering genomically imprinted genes, *in vitro* incubation of preimplantation mouse blastocysts with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, or dioxin) alters the pattern of DNA methylation in specific genes. *H19* and *IGF-2*, the affected genes, are also embryonically imprinted genes. TCDD, although not a ligand for the estrogen receptors ($ER\alpha$ or $-\beta$), has numerous associations with estrogen action at the molecular as well as functional levels, *e.g.* dioxin receptor (AhR) interacting with the $ER\alpha$ or $-\beta$ (23, 24) or TCDD-associated endometriosis in treated primates (25).

That epigenetic mechanisms may play a role in endocrine disruption helps explain the transgenerational effects of some hormonally active chemicals. Treatment with diethylstilbestrol (DES) during pregnancy results in vaginal adenocarcinoma in female offspring in humans (26) and mice (27). Female offspring of mice exposed to DES during pregnancy, when mated to control males, produce a second generation of females who, although not exposed to DES themselves, express this same rare genital tract cancer (8). This transgenerational transmission of a specific reproductive tract lesion would be hard to explain without invoking an epigenetic mechanism for heritable change and, given the finding of altered DNA methylation patterns in a specific uterine gene in mice treated developmentally with DES (4), we think a strong case can be made for such a conclusion. Newbold and colleagues (9) next showed that specific, rare genital tract cancers (rete testes cancers) are also expressed and therefore transmitted to the male offspring of females treated *in utero* with DES. In colloquial terms, this demonstrated the occurrence of reproductive tract tumors in the grandsons and granddaughters of mothers treated with DES. A possible mechanism for how DES exerts its action epigenetically has been proposed recently (28). The transmission of uniquely specific changes in the program of development in mice has implications for similarly exposed humans as well as the biology of hormonally induced disease.

It is likely that the transgenerational epigenetic effects seen with the environmental estrogen DES also occur with other EDCs. Rats treated with the estrogenic pesticide methoxychlor or the antiandrogenic fungicide vinclozolin during pregnancy produce male offspring that have decreased sperm capacity and fertility (6). Remarkably, the compromised fertility is passed through the adult male germ line for four generations with high penetrance. The authors demonstrated altered patterns of DNA methylation in the germ cells of generations two and three. Interestingly, individuals initially are fertile, but with age, fertility is reduced. This study is an elegant demonstration of epigenetic alteration in genes apparently important to reproductive function by two kinds of EDCs in an age-dependent manner.

Evolution

According to neo-Darwinian Modern Synthesis, natural selection of heritable variation results in small but cumulative genetic changes. This gene-based theory has served adequately for explaining extant species, but it does not entirely explain observed variation (29, 30). New developments in evolutionary theory, or what some have termed the Second Synthesis, consider the interplay of genes and the environment along with development, especially the emergent nature of development. However, most studies continue to be restricted to narrow windows of embryonic development or, at the most, to a single life-history stage. They do not take into consideration the cumulative nature of critical experiences throughout the life cycle. This requires giving careful thought to an additional dimension, namely the social environment in which the individual lives, which itself has a genetic basis that can evolve.

Morphological evolution results from mutation and selection such that gene-level changes are incorporated into the genome. Another mechanism may also exist, namely the epigenetic regulation of tandem repeat expansions and contractions that occur in the germ line during times of stress (20, 31). An example of epigenetic variation whereby the environment induces stable phenotypic changes without any genetic change is that of ectopic bristle outgrowth from the eye in *Drosophila* (18). Similar processes may occur in the evolution of other, interdependent complex traits. That is, the individual, with its adapted morphological, physiological, and behavioral traits, can be both a result and a cause of evolutionary change.

The ability of the genotype to produce different phenotypes in response to different environments has been termed plasticity. Commonly, the time of maximal plasticity appears to be during development; however, the individual's capacity to respond to environmental change or insult with heritable phenotypic variation at a later stage may also be possible. This ability to facilitate change has been termed adaptability (32). It is likely that suites of genes underlie the fundamental plasticity of an organism, particularly during development or life-history transitions. This characteristic of trait variability, whether molecular, cellular, physiological, morphological, or behavioral, represents the leading edge of evolution.

Epigenomic regulation of a network of genes and their products more accurately reflects the evolutionary process, particularly as it relates to the heritability of complex traits (29, 33). In this instance, new traits are constructed as the constituent genes, and their products interact in a temporal, spatial, and conditional (tissue or external environment) context (34). The adaptive responses that emerge in turn set the stage for future variation. In this manner, evolution can be a tandem process involving first development, with its built-in flexible responsiveness to both gene products and environment, followed by selection, which dictates which variants are spread and maintained (35). In this sense, it might be said that the "genome learns from its experience" (36).

How likely is it that EDCs and other environmental signals could actually contribute to the process of evolution? In this present context, it is the persistence of the challenge of EDCs

that yields to altered function over generations. For example, phytoestrogens (estrogenic compounds found in leguminous plants) can globally alter the pattern of methylation in mice exposed *in utero* (7). We have already mentioned that DES treatment of mice during development results in reproductive tract cancers, persistent up-regulation of key estrogen-responsive genes, and altered patterns of gene methylation in the affected genes (4) and that the cancer can be transmitted through two generations. In addition to DES, methoxychlor has been reported to increase global DNA methylation in uterine ribosomal DNA after *in utero* exposure; the alteration in methylation remains months after treatment (5). Recently, methoxychlor has been reported to alter the expression of *Hox* genes involved in the developmental patterning of the uterus (15). The finding that male infertility associated with prenatal exposure to the EDCs methoxychlor or vinclozolin in rats extends through four generations and is accompanied by altered methylation patterns in germ-cell DNA (6) suggests that EDCs have at least the potential for long-term change consistent with being a substrate for evolution. These and other studies provide insight into the mechanisms for epigenetic change that can be incorporated into a heritable system with functional significance in development.

A plausible scenario for the way in which EDCs may influence changes that lead to epigenetic inheritance is that EDC exposure might involve their ability to mimic the action of endogenous estrogens or by stressing the embryo by altering Hsp90 (20, 37). This in turn can result in methylation of imprinted genes during embryogenesis such that developmental pathways are affected. Consequent changes in DNA methylation patterns at CpG islands would be persistent and heritable. These CpG sites are often associated with 5' promoter regions of transcription factors and have a higher probability of undergoing mutation than other regions of the genome. But evidence suggesting that EDCs might reprogram methylation patterns that are then incorporated into the germ line, and hence transmitted to future generations, is still sparse.

This brings us to the role of behavioral plasticity in evolutionary change. Animals typically respond to changes in the environment by modifying their behavior, thereby changing their environment. Over generations, consequent modifications in anatomy, physiology, and patterns of gene regulation can arise out of the new environment's impact on the developmental processes associated with the new behavior. Such actions can be independent of mutational changes in the genotype. The "changes in behavior create the new variants on which natural selection works" (38). This is seen particularly clearly with sexual and reproductive behaviors (39). In nature, individuals (particularly females) actively discriminate and choose among potential mates; successful mating requires a reciprocal synchronicity in behavior and physiology among males and females (40). We know that EDCs influence the behavior of animals both in nature and in the lab (41, 42); might they also influence the embryo's developing nervous system such that, in adulthood, the individual's ability to discriminate and choose between possible mates is compromised? Extrapolating from altered behaviors to evolutionary significance is straightforward.

Endocrine Disruption

Endocrine disruption has been shown to be both acute (reversible) and long term, depending on the developmental stage at which the chemical is seen by the organism. Certainly, the acute effects of environmental estrogen in stimulating a target in an adult organism, such as vitellogenin induction by *p*-nonylphenol or *bis*-phenol A in male fish, frogs, reptiles, or birds (2) shows the power of environmental signaling. However, what is truly compelling are examples of permanent sex reversal such as that seen in turtles exposed early in development to PCBs (43) or the occurrence of pseudohermaphroditic polar bears in EDC-contaminated areas (44). There is a relationship between the developmental features of endocrine disruption and the evolutionary basis for this effect. This is a property of an evolutionarily ancient functional relationship between estrogen and the estrogen receptor.

We are struck by the conservation of certain portions of the ER α across metazoans, specifically the DNA-binding domain and, to a lesser extent, the ligand-binding domain. Moreover, analysis of the evolution of the nuclear receptor gene family suggests that this ancestral receptor is functionally similar to the ER (45), raising the possibility that the signaling motif is evolutionarily ancient. We are further struck by the widespread distribution of the functional signal interpreted as estrogen (46). The fact that animals, fungi, and plants have evolved a similar chemical signal that functions as estrogen suggests independent adaptations with separately evolved genes coding for proteins that serve a common function, namely recognition of a ubiquitous environmental compound. Numerous plants contain molecules that activate the vertebrate ER and induce an estrogenic effect in, for example, mice (2). At least one fungus also produces chemicals that fulfill the criteria to be considered estrogens. In fact, it has been shown that the chemicals in plants recognized by vertebrates as estrogens, the phytoestrogens, function as signaling molecules for transcription factors in rhizobial bacteria (47). The result of this symbiotic signal is the migration of bacteria into the root hair and the formation of root nodules with subsequent nitrogen fixation. Fox and colleagues (48) recently showed that environmental chemicals known to disrupt the vertebrate endocrine system would also disrupt the signaling system between alfalfa and *Rhizobium meliloti* at environmental levels. When looking at a wide range of pesticides and other environmental agents, this group was able to show that estrogenicity of the environmental agent was correlated with the disruption of the symbiotic signaling system (49); the ecological implications are obvious because this signaling system is the root of nitrogen fixation and opens a wholly new area of endocrine disruption. This suggests that the reason the function that we associate with estrogen may be so widely distributed in the environment and nature is because it is an environmentally ancient signal. Perhaps this is why most EDCs are estrogenic, not because estrogen is simply what has been looked for, but because it is the evolutionarily determined signal that is the most ubiquitous.

Health and Disease

In previous sections, we have examined the possibility that hormones or EDCs may function in evolution as epigenetic factors. Here we explore the epigenetic action of exogenous or environmental estrogens in health and disease. It may be that the processes are similar. The relationship of developmental change and evolution has been covered in an earlier section. We expand on that concept here by looking at the manner in which cell differentiation occurs, what role hormones may play, and what altering that process may do.

To understand the role of estrogens in development, there is hardly a more powerful model than that of the outcomes observed in humans and mice developmentally exposed to the synthetic estrogen DES. Female offspring of humans (26) or mice (27) exposed prenatally to DES have a risk for vaginal clear cell adenocarcinoma. The mechanisms underlying these developmentally induced lesions have been sought for three decades. There was the suggestion by clinical investigators that DES had altered the normal differentiation of the epithelial cells of the fetal cervix and vagina such that they responded abnormally to estrogen at puberty, because no cancers had been seen in prepubescent girls. Similarly, ovariectomy of developmentally DES-treated mice prevented the subsequent expression of uterine adenocarcinomas.

Epigenetic change in the molecular program of cell differentiation in the affected tissues may be a common mechanism. The clear cell cancers of the vagina in DES-exposed women displayed genetic instability consistent with epigenetic imprints in the absence of any expected mutation in classical oncogenes or tumor suppressor genes (50). Using a well validated mouse model for DES genital tract tumors, Li and colleagues (4) discovered that one of the estrogen-inducible genes in the mouse uterus, *lactotransferrin*, that had been shown earlier to be persistently up-regulated by developmental DES exposure, had an altered pattern of CpG methylation in the promoter region of the gene upstream from the estrogen response element. Subsequent work demonstrated that other developmentally up-regulated genes such as *fos* and *jun* also had persistent changes in the pattern of methylation of the gene after DES exposure during development (51, 52). These experiments raise the possibility that DES (and other environmental estrogens) alter the program of differentiation of estrogen target cells in the reproductive tract through an epigenetic mechanism.

Other studies support this hypothesis. In addition to cervicovaginal adenocarcinomas in female mice and humans exposed prenatally to DES and uterine adenocarcinoma in mice, it has been shown that developmental exposure to DES results in excess risk of uterine leiomyomas (fibroids) in mice (27), rats (53), and women (54). It was also recently reported that sea lions in areas contaminated with EDCs have a higher prevalence of uterine fibroids (55). The Eker rat carries a germ-line mutation in a tumor suppressor gene and is predisposed to uterine leiomyoma. Cook and colleagues (53) used this model system to demonstrate a DES-induced alteration in developmental imprinting as analyzed by tumor suppressor gene penetrance, concluding that developmental programming by estrogen works in concert with preexisting genetic change. In a population of 819 black and 504 white

women, fibroid status was determined by ultrasound screening or surgical record review, whereas prenatal DES exposure was determined by interview. DES-exposed women had a significantly greater risk for uterine fibroids and tended to have larger tumors. The authors conclude that their study, as well as animal studies, indicate a role for prenatal estrogen in the etiology of uterine leiomyoma in women (54).

In addition to the research linking EDCs, epigenetics, and reproductive disease at the molecular level, a growing body of information suggests that epigenetic effects might extend to gender differences in brain and behavior. Over 70 yr of research with animal models has demonstrated that sex hormones organize the brain perinatally such that the individual's sexuality is modified to determine how the individual's perception and learning abilities respond to nonsexual stimuli. Not only does the nature and amount of hormone affect individual development, but also the timing of this hormone exposure is important. However, although the principle of critical periods of hormone sensitivity is well established in animal studies, the data on human behavior are only now being collected (56). It is well known that conditions such as congenital adrenal hyperplasia, resulting in elevated androgen levels, influence subsequent gender identity and role (57). Even modestly higher *in utero* androgen exposure during fetal life has detectable effects in later adulthood. For example, a female dizygotic twin with a twin brother, as compared with a female dizygotic twin with a twin sister, exhibits more risk-taking behavior (58), has a more masculine pattern of cerebral lateralization (59), and has a more masculine pattern of aggression proneness (60). Psychosocial test scores of young adult women appear to be related to maternal levels of androgen and sex hormone binding globulin (SHBG) they experienced as fetuses (61, 62). That is, a significant portion of the variance among 250 women, aged 27–30 yr, in their gendered behavior can be accounted for by their exposure as fetuses during their mid-trimester of development to the androgens and SHBG in their mothers' circulation.

Waddington's model of a developmental landscape can also be applied meaningfully to the study of mental health disorders, namely that early developmental events can modify an adult individual's subsequent response to environmental and internal stimuli such that successive life-history events make the effect of the processes' underlying complex traits progressively harder to overcome. Indeed, Woolf (63) and Grossman and colleagues (64) have applied the concept of a developmental landscape to the understanding of the etiology of schizophrenia, fragile X syndrome, fetal alcohol syndrome, and depression. Both papers present compelling arguments that in psychopathology, early developmental events serve to canalize the individual into an ever-narrowing range of responses. It is significant that methylation has been implicated in the etiology of all of these disorders.

For many psychiatric disorders, there are significant gender differences in relative risk level and severity. In females, the incidence of eating disorders, major depressive disorder, obsessive-compulsive disorder, posttraumatic stress disorders (PTSD), anxiety and panic disorders, seasonal affective disorder, and Alzheimer's disease and dementia are 2-fold higher or greater than in men. Males are at higher risk for early-onset disorders such as autism and schizophrenia. That

these disorders may be modulated by hormones is suggested by the fact that the sex differences manifest after puberty or, in the case of schizophrenia in women, increasing symptoms are experienced during pregnancy and postpartum.

Might these gender differences in disease susceptibility, time of onset of symptoms, and drug response in mental health disorders be maintained in populations exposed to various EDCs, but overall risk levels increased? Alternatively, it is possible that the difference may be abolished, with male risk levels equivalent to that of females. This is an important question that deserves study. There are several reports indicating that both men and women exposed to DES *in utero* are more prone to depression compared with their unexposed siblings (65–67). Similarly, autism, a disorder more prevalent in males, has increased significantly in recent decades. New research suggests a relationship between high levels of fetal testosterone and autism (68). Aromatase inhibitors increase fetal testosterone, and some classes of endocrine disruptors are known to function as aromatase inhibitors such as the pesticide Roundup as well as some fungicides.

Thus, research indicates that factors such as stress, drugs, and hormones acting during embryogenesis can not only shape the subsequent behavioral phenotype of the individual but also modify the way the individual responds to adult experiences. The clinical significance of this work resides squarely within the concept identified as the fetal basis of adult disease, demonstrating, for example, that malnutrition in the mother during early pregnancy increases the risk in the child of schizophrenia in adulthood (69–73). These disorders are often precipitated by stress, which itself alters the endocrine state. Some of the women exposed directly to the World Trade Center collapse while pregnant developed PTSD. These women and their babies have lower cortisol levels compared with mothers who did not develop PTSD and their babies (74).

Building on a long history of research in developmental psychobiology, Meaney (75) has demonstrated that the nature and amount of care a pup receives from the mother modulates its reaction to stress later in life largely through effects on the glucocorticoid receptor (GR) in the hippocampus. This maternal effect can cross generations but is heritable only insofar as its manifestation depends upon the pup's experience in the first week of life. Recently, this group has documented that being reared by a high-quality mother results in the expression of NGFI-A, a nerve growth factor-inducible protein, that binds to the first exon of the GR gene, resulting in increased expression of GR (76). High-quality maternal care during this critical period results in the demethylation of NGFI-A and the acetylation of histones. Just as cross-fostering can reverse these molecular and behavioral changes, infusion of methionine, a histone deacetylase inhibitor, into the hippocampus can also reverse these events (77). Is there a counterpart in humans? Caspi and colleagues (78, 79) have demonstrated how the rearing environment can overcome the influence of genotype in the etiology of violent behavior.

Summary and Recommendations

Human health and disease is a complex interaction between the individual's genes and its environment factored over time. An interaction between an estrogen, for example,

and its receptor that may be normal and healthy in the adult may be catastrophic early in development as evidenced by the prenatal DES story. In the context of epigenetic programming and gene imprinting, we can now understand at a mechanistic level apparently irreversible changes in cell function that can lead to disease as a result of exogenous hormone exposures. We may even begin to understand how that same process may occur with endogenous hormones. Given that the process of gene imprinting is a normal one seen in cell differentiation and genomic allelic silencing, one may speculate that for estrogens or estrogenic chemicals, the distinction between physiology and pathology are blurred. It would seem to be important, then, to determine the epigenetic imprints associated with hormones in normal healthy cells and organs and compare these to the same patterns in diseased or dysfunctional ones. Similarly, endocrine changes underlie all major life-history transitions (e.g. puberty and reproduction). As individuals respond to these changes in internal state, they behave differently, thereby changing their environment. These altered behaviors can not only result from chromatin restructuring but also, at the same time, lay the foundation for perpetuating the environment that reinforces them. Given that patterns of imprints are usually the result of a network of signaling pathways that are often developmentally active, we again can see the role for evolutionary, developmental, and psychological thinking in human health (Fig. 1).

Thus, after many years of investigation, we are beginning to understand the mechanisms by which estrogens and other hormones, especially during development, can alter the genetic program of target cells without altering the sequence of DNA itself. This suggests a path to follow in exploring and ultimately

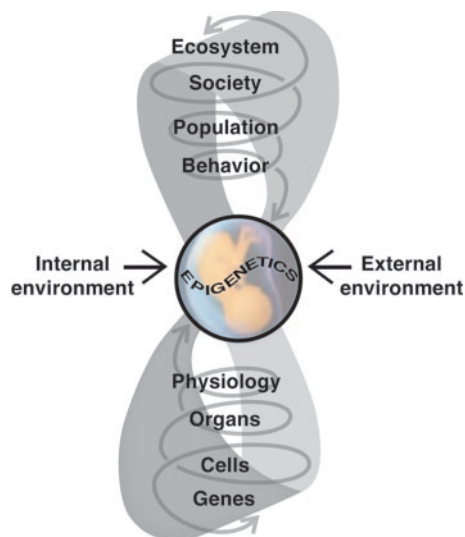


FIG. 1. The fetus is particularly vulnerable to changes in the external and internal environments, which interact to influence fetal development and have both immediate and life-long consequences. Such environmentally induced changes can occur at all levels of biological organization, from the molecular to the organism's behavior and place in society, and tend to be amplified in their consequences as they ascend through these levels. Ultimately, these influences may be epigenetic in nature, inducing mitotically heritable alterations in gene expression without changing the DNA.

coming to understand diseases of many organs and systems that may have environmental or endocrine origins.

Acknowledgments

We thank Christopher McLachlan for her acumen and perseverance in editing the manuscript, Tom Juenger for discussions, Andrea Gore for her editorial comments, and Gwen Gage for her rendering of the figure.

Received September 1, 2005. Accepted December 19, 2005.

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The preparation of this review was supported in part by National Institutes of Health Grants MH41770 and MH068273 (to D.C.) and Department of Energy Grant DE-FC26-00NT40843 (to J.A.M.).

References

1. Medawar P, Medawar J 1983 Aristotle to zoos. Cambridge, MA: Harvard University Press
2. McLachlan JA 2001 Environmental signaling: what embryos and evolution teach us about endocrine disrupting chemicals. *Endocr Rev* 22:319–341
3. Nijhout HF 2003 The importance of context in genetics. *Am Sci* 91:416–418
4. Li S, Washburn KA, Moore R, Uno T, Teng C, Newbold RR, McLachlan JA, Negishi M 1997 Developmental exposure to diethylstilbestrol elicits demethylation of estrogen-responsive lactoferrin gene in the mouse uterus. *Cancer Res* 57:4356–4359
5. Alworth LC, Howdeshell R, Ruhlen RL, Day JK, Lubahn DB, Huang TH-M, Besch-Williford CL, vom Saal FS 2002 Uterine responsiveness to estradiol and DNA methylation are altered by fetal exposure to diethylstilbestrol and methoxychlor in CD-1 mice: effects of low versus high doses. *Toxicol Appl Pharmacol* 183:10–22
6. Anway MD, Cupp AS, Uzumcu M, Skinner MK 2005 Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* 308:1466–1469
7. Dolinoy D, Weidman JR, Waterland RA, Jirtle RL, Maternal genistein alters coat color and protects *Ary* mouse offspring from obesity by modifying the fetal epigenome. In press
8. Newbold RR, Hanson RB, Jefferson WN, Bullock BC, Haseman J, McLachlan JA 1998 Increased tumors but uncompromised fertility in the female descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis* 19:1655–1663
9. Newbold RR, Hanson RB, Jefferson WN, Bullock BC, Haseman J, McLachlan JA 2000 Proliferative lesions and reproductive tract tumors in male descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis* 21:1355–1363
10. Waddington CH 1942 Canalization of development and the inheritance of acquired characters. *Nature* 150:563–565
11. Gilbert SF, Sarkar S 2000 Embracing complexity: organicism for the 21st century. *Dev Dynam* 219:1–9
12. Etinger, RS, Mambo, E, Evans, MI 1997 Estrogen-dependent transcriptional activation and vitellogenin gene memory. *Mol Endocrinol* 11:1985–1993
13. Andres, AC, Muellener, DB, Ryffel GU 1984 Persistence, methylation and expression of vitellogenin gene derivatives after injection into fertilized eggs of *Xenopus laevis*. *Nucleic Acids Res* 12:2283–2302
14. Huang WW, Yin Y, Bi Q, Chiang TC, Garner N, Vuoristo J, McLachlan JA, Ma L 2005 Developmental diethylstilbestrol (DES) exposure alters genetic pathways of uterine cytodifferentiation. *Mol Endocrinol* 19:669–682
15. Fei X, Chung H, Taylor HS 2005 Methoxychlor disrupts uterine Hoxa10 gene expression. *Endocrinology* 146:3445–3451
16. Waddington CH 1953 Genetic assimilation of an acquired character. *Evolution* 7:118–126
17. Cubas P, Vincet C, Coen E 1999 An epigenetic mutation responsible for natural variation in floral symmetry. *Nature* 401:157–161
18. Sollars V, Lu X, Xiao L, Want X, Garfinkel MD, Ruden DM 2003 Evidence for an epigenetic mechanism by which Hsp90 acts as a capacitor for morphological evolution. *Nat Genet* 33:70–74
19. Ellegren H 2000 Microsatellite mutations in the germline: implications for evolutionary inference. *Trends Genet* 16:551–558
20. Ruden DM, Garfinkel MD, Xiao L, Lu X 2005 Epigenetic regulation of trinuclotide repeat expansions and contractions and the “biased embryos” hypothesis for rapid morphological evolution. *Curr Genom* 6:145–155
21. Holliday R 1987 The inheritance of epigenetic defects. *Science* 238:163–170
22. Holliday R 1998 The possibility of epigenetic transmission of defects induced by teratogens. *Mutat Res* 422:203–205
23. Ohtake F, Takeyama K-L, Matsumoto T, Kitagawa H, Yamamoto Y, Nohara K, Tohyama C, Krust A, Mimura J, Chambon P, Yanagisawa J, Fujii-Kuriyama Y, Kato S 2003 Modulation of oestrogen receptor signaling by association with the activated dioxin receptor. *Nature* 423:545–550

24. Wu O, Ohsako S, Ishimura R, Suzuki JS, Tohyama C 2004 Exposure of mouse preimplantation embryos to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) alters the methylation status of imprinted genes *H19* and *Igf2*. *Biol Reprod* 70:1790–1797
25. Rier SE, Martin DC, Bowman RE, Dmowski WP, Becker JI 1993 Endometriosis in rhesus monkeys (*Macaca mulatta*) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Fundam Appl Toxicol* 21:433–441
26. Herbst AL, Ulfelder H, Poskanzer DC 1971 Adenocarcinoma of the vagina. Association of maternal stilbesterol therapy with tumor appearance in young women. *N Engl J Med* 284:878–881
27. McLachlan JA, Newbold RR, Bullock BC 1980 Long-term effects on the female mouse genital tract associated with prenatal exposure to diethylstilbestrol. *Cancer Res* 40:3988–3999
28. Ruden DM, Xiao L, Garfinkel MD, Lu X 2005 Hsp90 and environmental impacts on epigenetic states: a model for the trans-generational effects of diethylstilbestrol on uterine development and cancer. *Hum Mol Gen* 14:R149–R155
29. West-Eberhard MJ 2003 Developmental plasticity and evolution. New York: Oxford University Press
30. Jablonka E, Lamb MJ 1995 Epigenetic inheritance and evolution. Oxford, UK: Oxford University Press
31. Fondon 3rd JW, Garner HR 2004 Molecular origins of rapid and continuous morphological evolution. *Proc Natl Acad Sci USA* 101:18058–18063
32. Bateson P 2005 The return of the whole organism. *J Biosci* 30:31–39
33. Lewontin RC 2000 The triple helix: gene, organism and environment. Cambridge, MA: Harvard University Press
34. Kuo Z-Y 1967 The dynamics of behavior development: an epigenetic view. New York: Random House
35. West-Eberhard MJ 1998 Evolution in the light of developmental and cell biology, and vice versa. *Proc Natl Acad Sci USA* 95:8417–8419
36. Jaenisch R, Bird A 2003 Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* 33(Suppl): 245–254
37. Guerrero-Bosagna C, Sabat P, Valladares L 2005 Environmental signaling and evolutionary change: can exposure of pregnant mammals to environmental estrogens lead to epigenetically induced evolutionary changes in embryos? *Evol Dev* 7:341–350
38. Gottlieb G 2002 Developmental-behavioral initiation of evolutionary change. *Psychol Rev* 109:211–218
39. Crews D 2002 Diversity and evolution of neuroendocrine mechanisms underlying reproductive behavior. In: Becker J, Breedlove SM, Crews D, McCarthy M, eds. Behavioral endocrinology. 2nd ed. Cambridge, MA: MIT Press; 223–288
40. Hoskins CJ, Higgie M, McDonald KR, Moritz C 2005 Reinforcement drives rapid allopatric speciation. *Nature* 437:1353–1356
41. Crews D, Willingham E, Skipper JK 2000 Endocrine disruptors: present issues, future directions. *Q Rev Biol* 75:243–260
42. Zala SM, Penn DJ 2004 Abnormal behaviours induced by chemical pollution: a review of the evidence and new challenges. *Anim Behav* 68:649–664
43. Bergeron JM, Crews D, McLachlan JA 1994 PCBs as environmental estrogens: turtle sex determination as a biomarker of environmental contamination. *Environ Health Perspect* 102:780–781
44. Wiig O, Derocher AE, Cronin MM, Skaare JU 1998 Female pseudohermaphroditic polar bears at Svalbard. *J Wildl Dis* 34: 792–796
45. Thornton JW, Need E, Crews D 2003 Ancient origin of steroid receptors and reconstitution of the ancestral receptor. *Science* 301:1714–1717
46. McLachlan JA 1993 Functional toxicology: a new approach to detect biologically active xenobiotics. *Environ Health Perspect* 101:386–387
47. Mulligan JT, Long SR 1985 Induction of *Rhizobium meliloti* nodC expression by plant exudates requires nodD. *Proc Natl Acad Sci USA* 82:6609–6613
48. Fox JE, Starcevic M, Kow KY, Burow ME, McLachlan JA 2001 Nitrogen fixation. Endocrine disruptors and flavonoid signaling. *Nature* 413:128–129
49. Fox JE, Starcevic M, Jones PE, Burow ME, McLachlan JA 2004 Phytoestrogen signaling and symbiotic gene activation are disrupted by endocrine-disrupting chemicals. *Environ Health Perspect* 112:672–677
50. Boyd J, Takahashi H, Waggoner SE, Jones LA, Hajek RA, Wharton JT, Liu F, Fujino T, Barrett JC, McLachlan JA 1996 Molecular genetic analysis of diethylstilbestrol-associated clear cell adenocarcinomas of the vagina and cervix. *Cancer* 77:507–513
51. Li S, Hansman R, Newbold R, Davis B, McLachlan JA, Barrett JC 2003 Neonatal diethylstilbestrol exposure induces persistent elevation of c-fos expression and hypomethylation in its exon-4 in mouse uterus. *Mol Carcinog* 38:78–84
52. Iguchi T, Fukazawa Y, Bern HA 1995 Effects of sex hormones on oncogene expression in the vagina and on the development of sexual dimorphism of the pelvis and anococcygeus muscle in the mouse. *Environ Health Perspect* 103: 79–82
53. Cook JD, Davis BJ, Cai SL, Barrett JC, Conti CJ, Walker CL 2005 Interaction between genetic susceptibility and early-life environmental exposure determines tumor-suppressor-gene penetrance. *Proc Natl Acad Sci USA* 102:8644–8649
54. Baird DD, Newbold RR 2005 Prenatal diethylstilbestrol (DES) exposure is associated with uterine leiomyoma development. *Reprod Toxicol* 20:81–84
55. Backlin BM, Eriksson L 2003 Histology of uterine leiomyoma and occurrence in relation to reproductive activity in the Baltic gray seal. *Vet Pathol* 40:175–180
56. Cohen-Bendahan CC, van de Beek C, Berenbaum SA 2005 Prenatal sex hormone effects on child and adult sex-typed behavior: methods and findings. *Neurosci Biobehav Rev* 29:353–384
57. Hines M, Brook C, Conway GS 2004 Androgen and psychosexual development: core gender identity, sexual orientation and recalled childhood gender role behavior in women and men with congenital adrenal hyperplasia (CAH). *J Sex Res* 41:75–81
58. Resnick SM, Gottesman II, McGue M 1993 Sensation seeking in opposite-sex twins: an effect of prenatal hormones? *Behav Genet* 23:323–329
59. Cohen-Bendahan CC, Buitelaar JK, van Goozen SH, Cohen-Kettenis PT 2004 Prenatal exposure to testosterone and functional cerebral lateralization: a study in same-sex and opposite-sex twin girls. *Psychoneuroendocrinology* 29:911–916
60. Cohen-Bendahan CC, Buitelaar JK, van Goozen SH, Orlebeke JF, Cohen-Kettenis PT 2005 Is there an effect of prenatal testosterone on aggression and other behavioral traits? A study comparing same-sex and opposite-sex twin girls. *Horm Behav* 47:230–237
61. Udry JR, Morris NM, Kovenock J 1995 Androgen effects on women's gendered behaviour. *J Biosoc Sci* 27:359–368
62. Udry JR 2000 The biological limits of gender construction. *Am Sociol Rev* 65:443–457
63. Woolf CM 1997 Does the genotype for schizophrenia often remain unexpressed because of canalization and stochastic events during development? *Psychol Med* 27:659–668
64. Grossman AW, Churchill JD, McKinney BC, Kodish IM, Otte SL, Greenough WT 2003 Experience effects on brain development: possible contributions to psychopathology. *J Child Psychol Psychiatry* 44:33–63
65. Vessey MP, Fairweather DV, Norman-Smith B, Buckley J 1983 A randomized double-blind controlled trial of the value of stilboestrol therapy in pregnancy: long-term follow-up of mothers and their offspring. *Br J Obstet Gynaecol* 90:1007–1017
66. Fried-Cassorla M, Scholl TO, Borow LD, Strassman HD, Bowers EJ 1987 Depression and diethylstilbestrol exposure in women. *J Reprod Med* 32:847–850
67. Pillard RC, Rosen LR, Meyer-Bahlburg H, Weinrich JD, Feldman JF, Gruen R, Ehrhardt AA 1993 Psychopathology and social functioning in men prenatally exposed to diethylstilbestrol (DES). *Psychosom Med* 55:485–491
68. Knickmeyer RC, Wheelwright S, Taylor K, Raggatt P, Hackett G, Baron-Cohen S 2005 Gender-typed play and amniotic testosterone. *Dev Psychol* 41:517–528
69. Barker DJ, Eriksson JG, Forsen T, Osmond C 2002 Fetal origins of adult disease: strength of effects and biological basis. *Int J Epidemiol* 31:1235–1239
70. Barker DJ 2003 The developmental origins of adult disease. *Eur J Epidemiol* 18:733–736
71. Bateson P, Barker D, Clutton-Brock T, Deb D, D'Udine, B Foley RA, Gluckman P, Godfrey K, Kirkwood T, Lahr MM, McNamara J, Metcalfe NB, Monaghan P, Spencer HG, Sultan SE 2004 Developmental plasticity and human health. *Nature* 430:419–421
72. Gluckman PD, Hanson MA 2005 The fetal matrix: evolution, development and disease. Cambridge, UK: Cambridge University Press
73. St Clair D, Xu M, Wang P, Yu Y, Fang Y, Zhang F, Zheng X, Gu N, Feng G, Sham P, He L 2005 Rates of adult schizophrenia following prenatal exposure to the Chinese famine of 1959–1961. *JAMA* 294:557–562
74. Yehuda R, Engel SM, Brand SR, Seckl J, Marcus SM, Berkowitz GS 2005 Transgenerational effects of posttraumatic stress disorder in babies of mothers exposed to the World Trade Center attacks during pregnancy. *J Clin Endocrinol Metab* 90:4115–4118
75. Meaney MJ 2001 Maternal care, gene expression and the transmission of individual differences in stress reactivity across generations. *Annu Rev Neurosci* 24:161–192
76. Weaver IC, Cervoni N, Champagne FA, D'Allesio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ 2004 Epigenetic programming by maternal behavior. *Nat Neurosci* 7:847–854
77. Meaney JM, Szyf M 2005 Maternal care as a model for experience-dependent chromatin plasticity? *Trends Neurosci* 28:456–463
78. Caspi A, McClay J, Moffitt TE, Mill J, Martin C, Craig IW, Taylor A, Poulton R 2002 Role of genotype in the cycle of violence in maltreated children. *Science* 297:851–854
79. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R 2003 Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* 301:386–389