

Gene Expression and Environmental Exposures: New Opportunities for Disease Prevention

By John Peterson Myers, Ph.D.

Dr. Myers is founder and CEO of Environmental Health Sciences based in Charlottesville, Virginia, which publishes <http://www.environmentalhealthnews.org/>, tracking news about environmental health around the world. From 1990 to 2002 he directed the W. Alton Jones Foundation, guiding philanthropic investments in efforts to reduce the likelihood of nuclear war and to protect the global environment.

Decades of genetic research in medicine have established many links between diseases and genes, beginning with simple single-gene/unique-disease associations like phenylketonuria and sickle-cell anemia. Today new results are published almost weekly that reveal a genetic basis for yet another disease.

But what do these results mean?

The classic approach to the genetic basis of disease focuses on how differences in DNA nucleotide sequences contribute to disease susceptibility and causation via synthesis of aberrant proteins. This line of research has been spectacularly successful. Abnormal proteins resulting from gene mutations or different forms of alleles unquestionably can and do cause disease.

Yet careful epidemiological study of mutations that studies of heredity show are linked to disease usually reveals that only a small percentage of disease cases are actually attributable to the presence of the mutated gene in the patient. BRCA1 and breast cancer offer a typical example: Fewer than 10 percent of breast cancer patients possess the mutant form of the gene.¹

This common observation may reflect that susceptibility is controlled by a cluster of genes, of unknown number, with the promise that further research will ultimately reveal the identity of the responsible mutations that together cause the disease.

A well-established mechanism in molecular genetics, however, is emerging as the focus of increasing research that explores a different interpretation of what it means for a disease to be linked to a gene. Inappropriate gene expression—whether or not a gene is turned on or off at the appropriate time—can be just as

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important to disease susceptibility as whether the right form of allele is present in the first place. This different lens with which to view genetic disease is important because it opens up many possibilities for disease prevention, if the factors altering gene expression have environmental origins.

The common failure of epidemiological studies to reveal links between gene clusters and disease may reflect the role of altered expression of normal genes rather than the difficulties of teasing apart as yet undetected complex gene combinations.

For example, many women with breast cancer carry the normal form (i.e., DNA sequence) of BRCA1, but the gene is inappropriately silenced, and they therefore have abnormally low levels of a cancer-suppressing protein expressed by BRCA1.²

In this case, the immediate cause of BRCA1 silencing is DNA methylation, a mechanism involving the presence of a methyl group at the site on the gene where proteins normally bind to initiate gene expression. A similar suppression has been discovered for RASSF1A, another tumor-suppressing gene that when silenced via methylation is associated with breast and lung cancer.³

Methylation silences genes because the presence of the methyl group prevents the molecules that would normally switch genes on from reaching the gene's promoter site. Methylation is a common mechanism used by the cell's genetic machinery to control gene expression. It is crucial in normal development, helping guide tissue differentiation: Different genes are silenced in different tissues. Methylation of gene promoter sites affects a series of pathways crucial to normal development and homeostasis, including altered cell-cycle control, DNA damage repair, apoptosis and growth factor response.⁴

In the cases described above involving BRCA1 and RASSF1A, methylation is happening when and where it shouldn't. Changes in DNA methylation are now emerging as a major epigenetic mechanism leading to activation of oncogenes and deactivation of tumor-suppressing genes.⁴ In addition to breast and lung cancers, it has been noted in renal and colon cancers and acute lymphocytic leukemia. Multiple exogenous agents, including environmental contaminants and diet, alter DNA methylation.⁵

The control of gene expression has been a focus of molecular biology since classic experiments in the 1950s first began to explore how DNA fulfilled its hereditary role. What is new now are findings demonstrating that low-level exposures to a variety of agents, including environmental contaminants, can alter gene

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expression, affecting families of genes that are central to disease resistance, metabolic function, growth and development, et cetera.

Considerable attention has been focused within the last decade on changes induced by exogenous agents in the expression of genes under hormonal control. This work has revealed impacts at many of the control points in the chain of biochemical events that lead to protein synthesis. These impacts include:

- DNA methylation (see above). Multiple exogenous agents, including environmental contaminants and diet, affect DNA methylation.⁶
- Alteration of hormone concentration via up-regulation or down-regulation of enzymes involved in hormone synthesis. For example, the widely-used herbicide atrazine up-regulates aromatase activity, increasing the rate of conversion of testosterone to estradiol, thereby increasing estrogen levels.⁷
- Changes in hormone receptor density, which then change responsiveness to subsequent hormonal stimulation. For example, fetal exposure to the plastic monomer bisphenol A alters androgen receptor density in adult mice, increasing sensitivity to androgen stimulation.⁸
- Receptor binding by the exogenous agent, leading to up-regulation of gene expression for agents that successfully mimic the endogenous hormone, or down-regulation for antagonistic agents. For example, bisphenol A binds to the estrogen receptor, and that bound complex successfully increases expression of estrogen-responsive genes.⁸ In contrast, the DDT metabolite pp'-DDE binds to the androgen receptor, but the resulting complex does not induce gene expression; hence androgen-responsive gene expression is reduced in the presence of pp'-DDE because of competition for a limited number of receptors.⁹
- Alteration of the interaction between the bound ligand-receptor complex with transcription factors and the gene promoter site, preventing gene expression (see arsenic example, below).
- Activation of hormonally induced transcription factors, leading to gene expression (see bisphenol A example, below).

Two recent findings have added to the litany of control steps in gene expression that are vulnerable to exogenous interference: In the first, normal ligand-receptor binding occurs, but a contaminant alters the ability of the bound complex to initiate gene expression. Even at extremely low, noncytotoxic levels, arsenic interferes with glucocorticoid control of the tumor-suppressing gene

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phosphoenolpyruvate carboxykinase (PEPCK).¹⁰ In cells functioning normally, glucocorticoid enters the cell, it binds with its receptor (GR), and the resulting complex then moves within the nucleus and attaches to the gene's promoter site, initiating DNA transcription. In vitro experiments with a line of rat hepatoma cells show that arsenic present in concentrations as low as approximately 10 parts per billion disrupts transcription. While the mechanism remains to be confirmed, current evidence indicates that arsenic attaches to the bound glucocorticoid-receptor complex and prevents gene activation, perhaps through allosteric changes in GR. These findings are especially significant because of the low dose at which the effect is observable, levels found regularly in drinking water within the U.S., and because of other research establishing the importance of GR in mediating tumor suppression in skin and lungs, tissues that are susceptible to arsenic-induced cancers. They are also suggestive of the mechanism by which arsenic might act synergistically with other agents to induce cancers: When silenced by arsenic, GR is unable to activate PEPCK and suppress tumor formation initiated by other carcinogens.

Arsenic affects expression of many other genes also. A microarray analysis comparing gene expression patterns in liver tumors of adult mice with nontumorous liver tissue found dramatic overexpression of alpha-fetoprotein, c-myc, cyclin D1, proliferation-associated protein PAG and cytokeratin-18 in arsenic-exposed animals.¹¹ Gene expression changes persisted into adulthood despite the fact that arsenic exposure occurred only in gestation.

In the second example, bisphenol A binds with a nonclassical estrogen receptor on the surface of the cell membrane, initiating a series of biochemical events that lead to activation of the transcription factor CREB.¹² The plastic monomer is unexpectedly equipotent with estradiol at initiating the process, and effective at nanomolar levels. Other research on CREB activation shows it is a key control point in the expression of genes involved in adipogenesis,¹³ long-term memory formation,¹⁴ depression,¹⁵ apoptosis¹⁶ and other vital processes. Bisphenol A is found in human samples, including placental tissue, maternal blood and fetal blood, well within the concentration level effective in these experiments.¹⁷

There are two broad and legitimate responses to this growing understanding of the importance of disrupted gene expression in the etiology of disease. The prevailing response by medical researchers focused on this aspect of the genetic basis of disease is to search for pharmacological agents that can be used to manipulate gene expression, perhaps pushing the disturbed system back toward its undisturbed state, or adding a missing gene product. This response makes sense when no cause for the initial disruption has been identified and/or the focus is on patient treatment.

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The increasing number of examples of environmental factors that disrupt gene expression at low levels of exposure, however, raises a second approach, one that focuses on disease prevention. A high priority should be placed on identifying environmental agents that can disrupt gene expression even at extremely low levels, like arsenic and bisphenol A, and to begin to implement public health standards that reduce exposures. This approach has the considerable benefit of not risking further disruption by pharmacological agents of already perturbed systems. The side effects of agents affecting CREB activation, given the wide range of gene systems in which it is involved, may be far-reaching. It would be better to avoid the problem before it starts than to depend upon treatments that may have unintended consequences.

Given the wide array of health conditions now linked to altered patterns of gene expression—ranging from Parkinson's to obesity to immunosuppression and beyond—the future may hold in store opportunities for prevention through exposure reduction of many diseases never before linked to environmental exposures.

The full article with references is available from the CSA Office at (800) 345-3691 or write to andreadp@csahq.org.