Exploring Gene Expression in Sleep, Dreams and Hypnosis with the New DNA Microarray Technology: A Call for Clinical-Experimental Research

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Sleep, dream, hypnosis and awake states are generally recognized as a continuum of dynamical processes of the organism but current research has not resolved fundamental issues of how this continuum is to be explored. Recently the concept of Behavior State-Related Gene Expression has been introduced to describe how psychobiological states of awake, sleep, dreaming, arousal, novelty, environmental enrichment, physical exercise and stress are associated with different patterns of gene expression. New research techniques are emerging from The Human Genome Research Project that can be used to identify patterns of gene expression in such behavioral states of the organism on the cellular-genetic level. Current research developments in DNA Microarray Technology that are being used to rapidly assess gene expression in the various states of health and disease are discussed as a new approach to characterize the psychobiology of the cell cycle, arousal, stress, sleep, dream and the possible therapeutic applications of hypnosis. (Sleep and Hypnosis 2000;1:40-46)

Key words: sleep, dreams, therapeutic hypnosis, consciousness, memory, learning, novelty, psychobiology, behavior state-related gene expression, gene chip microarrays, DNA array technology.

INTRODUCTION

DNA Microarrays for Exploring Psychobiological States

It is a truism in science that the invention of a new instrument, technique or technology can revolutionize our understanding of nature. The telescope, microscope, EEG and now, in our own time, The Human Genome Project and the development of DNA microarrays is expanding human perception of our own nature far beyond what could have been imagined previously. DNA microarrays or gene chips consist of wafers of glass or other bonding surface about 1.5 centimeters square that appear to be analogous with the silicon chips of computer technology. Each chip is lined with thousands of microscopic wells or sites on which are attached short bits of DNA that can bond with any matching genes in a biological sample being studied. DNA microarrays may used to assess the expression and coordinated activity of as many as 10,000 genes in a single experiment.

An understanding of the new DNA microarray technology follows naturally from the basic structure and replication dynamics of the DNA molecule and the genetic code as described by the noble prize winning work of Watson and Crick. Genes are arranged in pairs that are zippered together on long strands of DNA arranged around each other in a double helix in chromosomes. During cell replication the double helix formed of the base pairing of nucleotides (i.e. A-T and G-C for DNA; A-U and G-C for RNA) comes unzipped momentarily until new bonds are formed. Molecular biologists have discovered how to arrange thousands of carefully identified small pieces of the unzipped DNA in a known order on a glass slide or "chip" with highly specialized robots. Each small piece of DNA (or oligionucleotide) can act as a molecular "probe." Such a probe is "sticky" and just waiting

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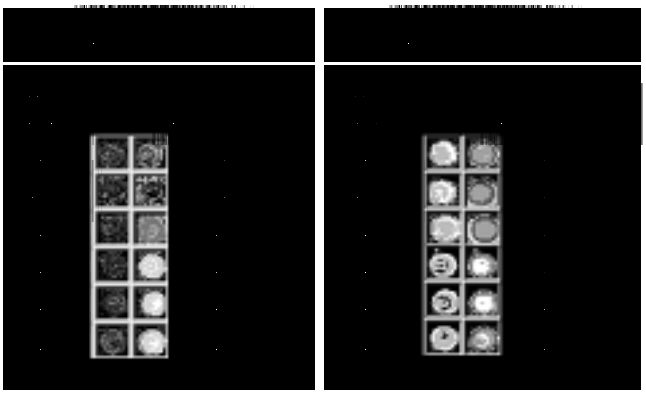
to attach or zipper itself to a corresponding piece of DNA that makes up a "target" gene in any solution that is incubated or "hybridized" with the probes on the chip.

It is now believed that we will eventually create DNA chips containing the entire human genome which was recently estimated to contain 142,634 genes (1). Such gene chips open the prospect of being able to identify the activity patterns of gene expression at any given moment in any condition or state of health or illness (2). For example, we can compare the differences in gene expression during cell replication versus a resting cell (3). We can explore the differences in gene expressions between a normal cell and a cancerous cells (4).

A variety of technologies for manufacturing DNA microarrays have been developed for these different applications and are known by many different names such as biochips, genome chips, cDNA arrays, DNA chips, Gene Chip Arrays, oligionucleotide arrays, high density microarrays etc. Figure one illustrates a typical experiment using DNA technology that has emerged from the National Human Genome Research Institute that can be used to identify patterns of gene expression in varying states of the organism on the cellular-genetic level. If we believe that sleep, dream, arousal and hypnosis are different states then we can expect DNA microarrarys will be able to identify the patterns of gene expression that are associated with these different states. This means that eventually we may be able to more precisely define exactly what we mean by arousal, sleep, dream and the various states of healing and hypnosis on a deep psychobiological level with such DNA microarrays.

Figure 1. Differential responses in two interlukin genes, as viewed in Incyte's GEM(TM) Tools software. The control (left image) for each gene is the O-hour treatment sample. (ND: not detectable)



Classical Mendelian Genetics and the New Functional Genomics: The Nature-Nurture Controversy in Hypnosis

To fully understand the significance of the DNA microarray revolution it may be important to first review the difference between Classical Mendelian Deterministic Genetics (that most people think of when they read about genes) and the Functional Genomics of Modern Molecular Biology that is generating the new DNA technology. The methods of early classical Mendelian genetics usually studied genetic determinism: the dynamics of how the inheritance of dominant and recessive genes determine certain phenotypic traits of the organism that are easy to observe such as eye, hair or skin color. The basic theory of Mendelian genetics - or perhaps we should call it "the basic dogma" - was that one or at most a few genes determined one biological or behavioral trait. According to this early dogma nature alone determined such physical traits; nurture or life experience supposedly had nothing to do with such biologically determined traits. It was from this basic dogma that the nature-nurture controversy arose when we attempted to extend classical Mendelian genetics to the study of human behavior. Many statistically oriented studies on animals and human twins documented how intelligence, for example, was related to the inheritance of genes in a deterministic manner. This led to the belief that intelligence and by implication many other human traits and behaviors were determined entirely by nature and could not be changed.

While such traditional Mendelian studies of one-gene-one-function have demonstrated that genes and behavior are related, these studies are slow, tedious, expensive and difficult to replicate. What is worst such studies have given rise to the grave misconception that we can identify one or a few genes as the ultimate cause of every human illness and behavioral problem: fix the gene with some sort of chemical manipulation and the problem is solved. This oversimplified and erroneous point of view ignores the broader truth that most genes are expressed (that is, turned on and off) in coordinated patterns of activity in response to signals from the extracellular environment (5). The new functional genomics of molecular biology focuses on these broad patterns of gene expression rather than the one- gene-one-function or trait approach of early Mendelian genetics. The new DNA microarray technology of functional genomics enables us to formulate a dynamic picture of the coordinated activity of hundreds of genes in a single experiment exploring basic life processes and the fluctuating states of the organism as it interacts with its environment in an adaptive and creative manner. In a recent technological symposium, for example, Conklin (6) reported that as many as 600 genes were differentially expressed in cardiomyopathy with the new DNA microarray technology. Similarly, several hundred genes were involved in the assessment of the effects of chronic, high glucose exposure (7). The study of such patterns of gene expression in changing life conditions is one as-

pect of the newly emerging field of bioinformatics (8). The early experimental methods of molecular biology, in imitation of the early dogma of Mendelian theory, usually studied the relationship between one gene and one biological or behavioral function. These studies identified a number of genes associated with states of arousal, sleep, and dreaming (9) in what has been called, "Behavior State-Related Gene Expression" (10-12). A recent review of the genetics of sleep and its disorders has identified genes associated with states of arousal, sleep, dreams (REM sleep), narcolepsy-cataplexy, sleepwalking, sleeptalking, sleep terrors, sleep apnea and related sleep disturbances (13). While the dissociative aspects of such disturbances have been associated with hypnosis in the historical literature, it is not yet known whether these associations imply that hypnosis is related to varying patterns of gene expression as well.

More recent studies on the Functional Genomics of Modern Molecular Biology indicate that human intelligence, neurogenesis and the growth of the brain is plastic or changeable as a function of novel and enriching life experience that can evoke many complex and coordinated patterns of gene expression (12,14,15). Life experience cannot change the biological inheritance of genes as studied by classical Mendelian genetics. Life experience can, however, modulate the expression of a certain proportion of genes (sometimes called "housekeeping genes") throughout the life cycle. That is, intelligence and many other human psychobiological functions are related to nurture (flexible changes in gene expression) in response to ongoing changes in life experience as well as nature (biological determinism of Mendelian gene inheritance) that was fixed once and for all for each individual during sexual reproduction and conception.

An approach toward the resolution of the nature-nurture controversy is now possible with the recognition of this crucial conceptual difference between the deterministic inheritance of all genes versus functional expression of some genes in response to changing life conditions. There is truth on both sides of the controversy. In the field of hypnosis, for example, the deterministic or nature side of the controversy was championed by researchers such as Hilgard (16) who found that the trait of hypnotic susceptibility as measured by standardized hypnotic susceptibility scales was apparently fixed and unchanging throughout the life cycle. Other researchers such as Erickson (17) championed the flexible or nurture side of the controversy by demonstrating, by contrast, how the experience of hypnosis was changeable as a function of ongoing experience in clinical settings as well as real life.

The application of the new DNA microarray technology now promises to deepen our understanding of both the nature and the nurture aspects of hypnotic experience. If hypnosis is a special psychobiological state (15,18) it would now be possible to take a sample of a human subject s blood, for example, and determine which genes were expressed (turned on) during a hypnotic session and which genes were turned off or not affected at all by hypnosis. Can hypnosis, the placebo response and the many methods of alternative and complementary medicine really facilitate real, physical healing as has been purported since ancient times (19)? Most statistical "outcome studies" attempting to deal with these issues have remained controversial because statistical correlation does not prove causation. Such outcome studies have not elucidated any scientifically accepted mechanism by which such healing could take place. DNA microarray technology now provides the possibility for dealing with this controversial issue in an experimentally verifiable manner. First let us identify the specific patterns of gene expression associated with any psychosomatic or physical illness. Let us then compare this gene expression list during illness with the list of gene expression associated with the various approaches to therapeutic hypnosis. It then would be a straightforward task to determine whether hypnosis or any other method of alternative/complementary medicine actually modulates gene expression in the direction closer to the patterns found in normal or optimal health. With such prospects in mind let us now take a look at some model experiments with DNA microarrays that illustrate how they could be used to explore the awake, sleep, dream and hypnosis continuum together with their therapeutic possibilities.

Model Experiments with DNA Microarrays

1. The Ultradian Periodicity of the Immune System

An instructive example of the use of the new DNA microarray technology to study the periodicity of the immune system and the cell cycle is available on the internet (http://gem.incyte.com/gem/data/unigemv/index.shtml). We choose to review these examples of DNA microarray technology because they illustrate the ultradian time parameters of fundamental life processes such as the immune system that have been proposed as the ultimate psychobiological basis of therapeutic hypnosis, placebos and a host of other approaches described as alternative or complementary medicine (15,19). Ultradian time parameters refer to life processes requiring less than 20 hours in contrast with Circadian time parameters that require 24 hours. Kleitman's Basic Rest Activity Cycle (BRAC) of 90-120 minutes is a basic ultradian life process characteristic of rhythms of awake, sleep, REM dreams,

and hypnosis as well as cellular-genetic processes at the molecular level (20).

Incyte researchers designed a model experiment to illustrate the real time dynamics of the immune system with the use of their DNA microarrays (UniGEM V) that can quantify the expression of more than 7000 genes at one time. They isolated peripheral blood mononuclear cells (PBMC) from healthy volunteers who included many of the major cell types of the immune system such as monocytes, natural killer cells, dendritic cells, T and B-lymphocytes. These cells express the common housekeeping genes responsive to extracellular signaling as described above as well as many tissue specific genes such as cytokines, transcription factors and membrane receptors. These PBMCs were stimulated with the mitogens PMA and ionomycin in vitro and gene expression was assessed after 0.5, 1, 2, 4, and 8 hours of activation. As illustrated in figures 2 and 3 there were significantly different changes in the expression of two interleukin genes that are known to respond differently to PMA and ionomycin.

Figure 2. Interlukin 2 and 10. The levels of interlukin 2 mRNA detected by the UniGEM V microarray dramatically rose over time; levels of interlukin 10 mRNA remained stable throughout the activation.

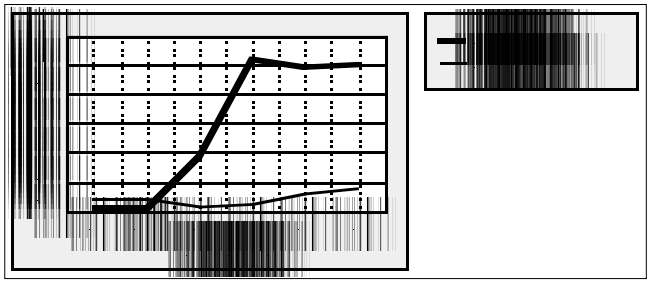
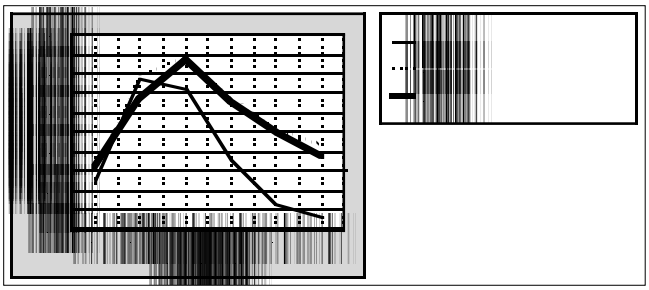


Figure 3. The cluster regroups the Early Growth Response (EGR) 1,2 and 3 genes only. The expression of EGR 1,2, and 3 genes in PBMC was significantly up-regulated after 30-minutes of activation with PMA and ionomycin. This gene expression induction peaked between 0.5 and 1 hour for all three genes and subsided at later time points.



This model experiment in the use of DNA microarrays is particularly interesting because it confirms how this new technology can be used to easily and comprehensively replicate and greatly expand the one-gene-one-functional response approach of classical molecular genomics. Glaser et al (21,22), for example, found in a series of one-gene-one-response experiments that the interleukin-2 receptor gene was suppressed in medical students while experiencing the psychosocial stress of medical exam week. The interleukin-2 gene also plays an important therapeutic role in strengthening the human immune system's signaling capacity in the medical model of cancer therapy (23). Taken together these experiments indicate that any medical or alternative therapeutic method (e.g. Hypnosis, the relaxation response, meditation, biofeedback, therapeutic touch etc.) that purports to enhance the functioning of the human immune system could be assessed rapidly and effectively in a scientific manner with the new DNA microarray technology. DNA microarrays could be used as a universal measure to assess the effectiveness of the molecular medical model of western medicine as well as the traditional healing approaches of many cultures throughout human history.

The Ultradian Periodicity of the Cell Cycle

Figures 4-7 illustrate the ultradian time parameters of cell cycle dynamics in response to extracellular signaling of PBMCs by PMA and ionomycin in vitro by the Incyte research group as described above. Figure 4 is particularly interesting because it illustrates the ultradian time parameters of immediate early genes that have been associated with the continuum of arousal between awake, sleep and REM dreaming (12,24). Figures 5-7 illustrate a variety of other ultradian time parameters in the intermediate and late activated ranges of gene expression in response to extracellular signaling. These are the basic ultradian time parameters that are associated with a wide variety of other biological processes such metabolism, homeostasis, cell division, growth and healing (12,15). These same ultradian time parameters are found in significant psychobiological processes in response to novelty, environmental enrichment and physical exercise leading to neurogenesis in the human brain (25). Interestingly these same ultradian time parameters are found in the expression of the Zif-268 gene during REM sleep after exposure to a daytime experience of "memorable" environmental events (26). Sexual behavior and many psychiatric problems such as pain, traumatic stress, drug addictions, psychosis, and psychosomatic disorders are expressed within the same time parameters (12,15). A number of reviews have explored these ultradian time parameters in the therapeutic applications of hypnosis and imagery (18).

Is this similarity between the time parameters of fundamental life processes at the cellular-genetic-molecular level illustrated in figures 2 through 7 and the therapeutic applications of hypnosis a simple coincidence or is there a direct causal link between the two?

Figure 4. The cluster shown regroups genes from different families, which all demonstrated a rapid up-regulation upon activation of PBMC with PMA and ionomycin and a subsequent return to baseline expression. Unlike genes regrouped in Figure 3, the genes regrouped in Figure 4 displayed a wider range of time at which the expression peaked (between .05 and 2 hours).

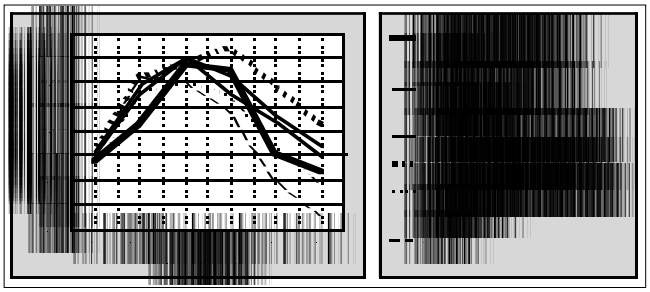


Figure 5. The cluster regroups genes significantly up-regulated between 1 and 2 hours of activation, displaying a peak expression between 2 and 4 hours of stimulation. In addition, these genes remained highly expressed after 8 hours of activation.

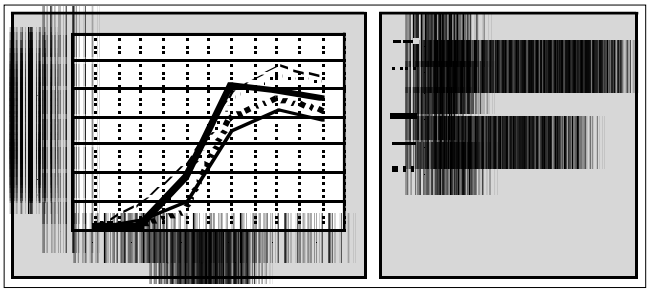


Figure 6. The cluster regroups genes significantly up-regulated between 2 and 4 hours of activation, displaying a peak expression between 4 and 8 hours of stimulation.

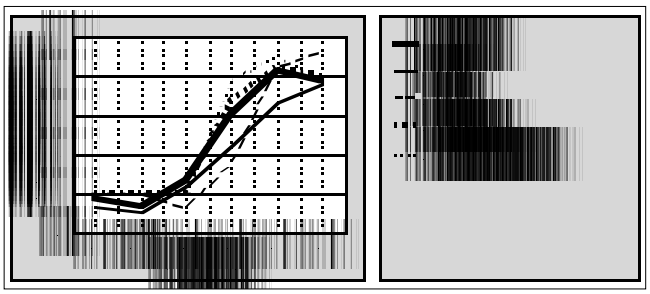
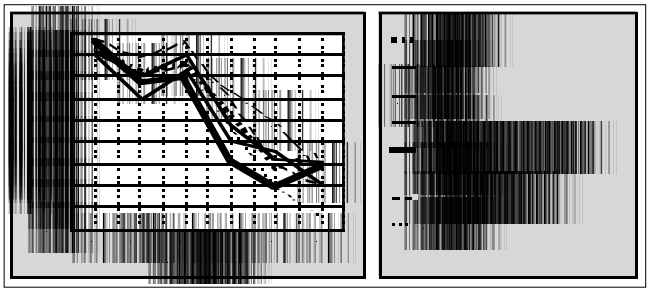


Figure 7. The cluster regroups genes significantly down-regulated after 2 hours of activation and beyond. Interestingly, except for the "human clone 23815" gene, the genes belonging to this cluster displayed a moderate decrease in their expression at the 30-minute time point (but this negative trend was partially reverted at the 1-hour time point).



Current experimentation and data are not sufficient to answer this question. More direct tests of gene expression during stress, trauma and illness and the purported psychobiological healing effects of hypnosis are needed. How could such direct tests be carried out?

After a lifetime of clinical practice Ewin (27), an emergency room surgeon and a former president of The American Society of Clinical Hypnosis, documented how hypnotic suggestions for cooling administered within two hours (a typical ultradian time BRAC) of a severe burn can reduce inflammation and facilitate healing to a much greater degree than when hypnotic suggestion is used more than two hours after the burn. Research is now needed to assess the hypothesis that the mechanism of this therapeutic application of hypnosis may be in blocking the patterns of gene expression that mediate inflammation via the formation of "stress proteins" whose over-production after two hours complicates the healing process in burn patients. Figures 3, 6 and 7 above imply that Ewin's hypnotic suggestions may be operating on the expression of intermediate and/or late activated genes. These model experiments illustrate the rich potential harvest of the Human Genome Project and DNA microarrays for exploring and integrating the entire range of human behavior from the molecular-genetic level to the emergent dynamics of physical healing mediated by hypnosis. DNA microarray technology provides us with opportunities to directly test the relevance of hypnosis and the wide variety of behavioral approaches involving consciousness and psychosocial processes to medicine, healing and health (20).

Stress and the Aging Process

Human stress has be explored on a variety of different levels with a wide variety of different techniques leading to different therapeutic efforts to ameliorate stress ranging from psychoanalysis, hypnosis, meditation, shamanistic and spiritual processes to biofeedback, body therapies and the new medical model of medicine at the level of functional genomics. Is there any common denominator to all these different theories and approaches to stress? The model experiments presented here imply that the dynamics of homeostasis and adaptation at the level of gene expression that are signaled and activated by stress-stimuli from the outer environment is this common denominator. One of the most interesting series of studies illustrating the cellular-genetic dynamics of stress used DNA array technology to study the changing patterns of gene expression during the aging process (28). These researchers found significant differences in gene expression patterns of the gastrocnemius muscle of adult (5 months old) versus aging mice (30 months old) "indicative of a marked stress response and lower expression of metabolic and biosynthetic genes." Further they

found that "Most alterations were either completely or partially prevented by caloric restriction, the only intervention known to retard aging in mammals." It is significant to note that one of the stress-related genes expressed in aging mice is translated into the production of Heat Shock 27 kDa Protein. We now need to assess the more specific hypothesis that healing in burn patients with Ewin's hypnotic suggestions will find that Heat Shock 27 kDa Protein is one of the "stress proteins" whose over-production is prevented when therapeutic suggestions are administered within two hours of receiving the burn trauma and stress.

Internet Access to DNA Microarray Technology and Research

DNA microarray technology and research is so new and changing so rapidly that it is almost impossible to stay current with the traditional venues of scientific publication such as books and journals. Most researchers working with this new technology are active in communicating their findings on the world wide web of the internet. It will therefore be of value to list here some of the more interesting and significant internet sites that can help readers orient themselves to the new possibilities of DNA microarray technology and research.

http://www.Gene-Chips.com is the most general and comprehensive web site where one can find listings of the design and sources of DNA microarray systems, review articles on DNA array technology, links to academic research papers and industrial laboratories as well as forthcoming workshops and meeting. Most of the other sites mentioned in this paper were found on this well updated site.

http://library.genetics.nature.com/server-java/Propub/genetics/ng0199supp.contents This site contains 10 review articles on DNA microarray technology and research that can be downloaded free.

http://www.ornl.gov/hgmis/ An excellent site for a general orientation of the current state of government supported DNA microarray research.

http://www.wenet.net/~telechem/DNA-Microarray-Protocols/ An excellent model for the design an execution of DNA microarray protocols.

http://gdbwww.gdb.org/ The Genome DataBase containing detailed information on an international research of The Human Genome Project.

http://bioinformatics.weizmann.ac.il/cards-bin/cardsearch.pl One of the best comprehensive sources of systematic information on individual genes and the emerging science of bioinformatics.

Summary

The Human Genome Project is providing unexpected benefits in the development of DNA microarray technology for exploring the continuum of awake, sleep, dream and hypnosis states. DNA microarray technology opens the possibility of answering many fundamental questions such as the following about the nature-nurture controversy and the deep psychobiology of healing with behavior state-related gene expression in a scientific manner. If environmental stress modulates gene expression across the continuum of arousal, sleep and dreaming may we conclude that any procedure that reduces stress also modulates gene expression? If one decides to relax and go to sleep, for example, does this mean that one is using conscious cognitive decisions to modulate human behavior and its associated patterns of gene expression? Likewise, If one makes a conscious decision to exercise and/or engage in novel experiences does this mean that humans have the capacity to consciously optimize neurogenesis and modulate gene expression in the various cells of the brain and body? If one uses hypnosis to facilitate such behavioral states does this imply that hypnosis is being used to modulate the psychobiology of human experience at the molecular level of gene expression?

If we believe the answer to these questions is "yes" then there are profoundly important implications for the future of research on the psychobiology of the continuum of awake, sleep, dreaming, therapeutic hypnosis and related states with DNA microarrays. If most human psychobiological states of health and illness are characterized by different patterns of gene expression then we may hypothesize that if hypnosis is a specific psychobiological state, for example, it too will be characterized by distinct patterns of gene expression. We have reviewed how the new DNA microarray technology now emerging from The Human Genome Project makes possible a variety of scientific models outlining how we could test this deep psychobiological theory of therapeutic hypnosis and related approaches to facilitating healing on the cellular-genetic-molecular level.

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