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Commentary

Mechanogenomic Control of DNA Exposure and Sequestration

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There is longstanding recognition and appreciation for striking differences in the structure, assembly and organization of regulatory machinery in normal and cancer cells. Drawings from 100 years ago of Galeotti's comparisons of cancer cell and normal cell chromosomes illustrated gross differences in chromatin structure and chromosome numbers in human tissue harboring a tumor.[1] Tumor-related perturbations in nuclear structure-function interrelationships are well documented by prominent perturbations in the size and number of nucleoli, indicating the reconfiguration of the nuclear infrastructure that supports ribosomal gene expression and consequently protein biosynthesis. However, how higher order chromatin structure is controlled and maintained by the cancer cell and its microenvironment, compared to normal cells, has remained beyond our reach. The work presented by Maniotis et al[2] in this issue of *The American Journal of Pathology* shows there are striking differences in restriction enzyme sensitivity between tumor cell and normal cell genomes. Furthermore, Maniotis et al propose that the mechanical contiguity and coordination of nuclear proteins bound to DNA, the cytoskeleton, and the extracellular matrix (ECM) may work in concert to provide a cytoarchitectural resistance mechanism deep within the cell. When verified in other laboratories, these findings may constitute the basis for a paradigm shift in the way we view how the genomes of higher eukaryotic cells, and malignant cells in particular, are regulated.

By using DNase digestions and nick-end labeling techniques, a previous generation of tumor biologists had established that in order for a gene to become expressed, it had to be "exposed." Moreover, Puck et al[3] had shown that transformed and tumor cells that were "reverse transformed" to a normal phenotype with various chemical compounds exhibited a shift in their nuclei's sensitivity to DNase I, and exhibited profound changes in their cytoskeletons and overall morphology.

Maniotis et al[2] have compared the extent of exposure or sequestration of a well-characterized collection of highly invasive, poorly invasive, and normal human cell genomes to digestion by specific restriction enzymes. Restriction enzyme digests performed on intact nuclei, not treated with any other chemical agents, showed that *Alu*I and *Msp*I both distinguished normal cell genomes from tumor cell genomes. For example, highly invasive cell nuclei always resisted digestion with *Alu*I and *Msp*I compared to poorly invasive or normal cells in permeabilized cell models, in cell smear preparations, in suspended cells using flow cytometry, and in touch preps of human tissue. Specificity is suggested because other restriction enzymes did not discriminate among normal or tumor cell genomes.

Differences in Alu site exposure were shown to be independent of the cell cycle, as shown by comparing digestions of complete sets of mitotic chromosomes[4,5] with digestions of interphase nuclei from the same cell types. Taken together with other restriction sites that exhibit cell cycle-stage specific accessibility[6–8] it appears that there are both conserved and transient components of chromatin organization that must be accommodated during the cell cycle period. It is necessary to mechanistically explain parameters of mitotic chromosome condensation that are independent from the sequestration of restriction sites along the DNA, and the observation that nuclear matrix-associated AML[9–11] and ALL[12,13] phenotype-restricted transcription factors, as well as UBF-1[14] remain on target gene loci during mitosis. New models are required to explain higher order chromatin structure more comprehensively. Such models should account for components of cell cycle-driven chromatin condensation that are operative independent of how specific restriction enzyme sites are cryptic or exposed in highly invasive, poorly invasive, and normal cells.

Experiments were also presented in the study that showed exposure of chromatin or chromosomes to DTT or β-mercaptoethanol rendered highly invasive and sequestered cell genomes sensitive to *Alu*I. This suggests that proteins rich in disulfide bonds are involved in the sequestration phenomenon in highly invasive cell nuclei.

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Enzymes or reagents that remove histones or topoisomerases[5] did not discriminate among genomes tested from different cell types. These observations are consistent with the idea that alterations in higher order chromatin structure and may, in part, be controlled by disulfide-rich nuclear matrix proteins,[15–21] which orchestrate widespread changes in restriction enzyme site exposure and sequestration. However, these digestion experiments also suggest that the disulfide-rich nuclear matrix cannot be a static or fixed structure, which is consistent with current models of chromatin structure and higher order nuclear organization of gene regulatory machinery in nuclear matrix-associated microenvironments that support combinatorial control of gene expression by dynamic, physiologically responsive targeting and retention mechanisms.[22–34]

Because digestions of DNA in nuclei of normal cells with *Alu*I and *Msp*I appear to additionally separate nucleoli from structural components of the nucleus, it appears that the DNA within normal cell chromatin, when it is not digested, may impose constraints that contribute to the architectural organization of intranuclear structure. Maniotis' case[2] for DNA as a principal scaffolding on which nuclear proteins are bound, and not vice versa, is consistent with his previous observations of nuclease digestions of microsurgically isolated mitotic chromosomes derived from normal endothelial cells and fibroblasts,[4] and is supported by his observation that complete chromosome sets are produced as intact genomes from both normal mitotic cells and mitotic tumor cells via microsurgical removal of any single chromosome.[4,5] Reversal of the relative insensitivity of chromatin of highly invasive cells to *Alu*I by disulfide-disrupting agents suggests that the sensitivity of invasive nuclei to *Alu*I and disulfide-rich protein disruption may be due to increased representation of these disulfide-rich proteins, or may suggest that there is a higher affinity of these proteins for the DNA of highly invasive cells. Further work is needed to determine whether nicking the DNA with *Alu*I leads to discontinuities along the DNA, which in turn permits DNA supercoiling to unravel sufficiently to dissociate the disulfide-rich proteins from the DNA throughout the genome, and allow complete digestion to occur.

There is a requirement to characterize the nuclear matrix disulfide-rich proteins that may be facilitating architecturally linked control of gene expression. Equally important is the necessity to relate the involvement of DNA as a scaffold for control of gene expression that differs in normal and cancer cells with the growing evidence for autologous intranuclear trafficking of regulatory proteins[11,32–34] that are key for organization and assembly of the regulatory machinery for context-dependent, combinatorial control of gene expression in nuclear matrix-associated microenvironments of interphase cells and at chromosomal loci of mitotic cells.[35–43]

In the second part of the work presented by Maniotis et al[2] in this issue, a systematic search was initiated to test if growth factors, or soluble and polymerized ECM molecules, affect the sequestration or exposure of DNA, and to test if there was specificity of different ECM molecule types on Alu site exposure or sequestration. Exposure of living cells to soluble laminin and RGD-C resulted in sequestration of Alu sites from digestion by *Alu*I in all cells tested. However, sequestration induced by Matrigel, laminin, and RGD-C was always more intense in the nuclei of cells of increasing grades of invasiveness. Serum, fibronectin, bFGF, EGF, and type I collagen had no effect on the seqestration of *Alu*I sensitive sites. When cells were grown in or on polymerized Matrigel or laminin platforms, profound sequestration was observed. This was in contrast to cells that were situated on Type I collagen, serum, or fibronectin. In addition, when comparing six repeated microarrays of highly invasive cells situated on Matrigel versus the same highly invasive cells not situated on Matrigel, a consistent differential expression of 990 transcripts was obtained. These results, when taken together with the observed chemical changes in sensitivity underlying the nuclease digestions, provide preliminary evidence that exposure of only one extracellular matrix molecule on the cell surface for 30 minutes can induce global effects on chromatin organization.

In multicellular organisms, the cytoskeleton and extracellular matrix are known to play a fundamental role in determining cellular behaviors. To test if different cytoskeletal fiber systems influenced the sequestration or exposed the Alu sites, a variety of cytoskeleton-disrupting drugs were used by Maniotis et al[2] to determine whether the higher order structure of chromatin was controlled by actin, microtubules, or intermediate filaments. Maniotis et al[44] had previously shown that a tug to an integrin receptor could alter the molecular alignment of intranuclear molecules in 1 second, and that each cytoskeletal system exerted a different stabilizing effect on both nuclear structure, and force transduction. In this study, each cytoskeletal fiber system profoundly contributed to the sequestration or exposure effect. Actin disruption decreased sequestration, while microtubule or intermediate filament disruption dramatically increased sequestration. In these experiments, the data suggest that nuclear size and cytoplasmic spreading and gene sequestration or exposure are co-events. A similar relationship between cell growth, cell "differentiation," and nuclear size in endothelial cells was shown long ago by Folkman and Moscona[45] on monolayers coated with increasing amounts of a cellular adhesive. The results of the Maniotis et al study[2] extend these observations to include the sequestration and exposure of specific DNA sites, and a compelling and testable model is advanced that involves cytoskeletal control of nuclear structure and nuclear pore complexes.

Together, these findings raise, and experimentally address, new questions regarding the fundamental way genetic information is controlled by proteins containing disulfide-rich bonds, by the extracellular matrix microenvironment, and by the cytoskeleton. The work also has contributed several new approaches that can be used as diagnostic tools. Furthermore, the observed differences in the sequestration of DNA among cells of varying invasive behavior can be exploited to distinguish differing degrees of malignancy that do not depend on presently used molecular markers. Therefore, the differential sensitivity of Alu sites in highly invasive tumor cells versus

noninvasive tumor cells offers an alternative for dependence on molecular markers, which have been shown to be highly variable in the context of vasculogenic mimicry, and which manifests as molecular mimicry (deregulated protein expression) in tissue sections of the most invasive types of tumors.[46–48] For example, the most highly invasive melanoma tumor cells, although they ultimately derive from the neural crest, are able to express protein markers characteristic of a variety of different normal cell types (such as endothelial cells), but also markers specific to epithelial cells, and a wide variety of other cell types, depending on the degree of deregulation. In this context, clinically relevant lessons can be learned about mechanisms for drug resistance that are mediated by microenvironments that are key components of tissue organization.[49,50]

All considered, it appears that how genes are sequestered, exposed, and expressed in higher eukaryotic cells can be viewed as part of a mechanically coordinated, cell shape-dependent, hierarchical system.[49,51–61] In this regard, it has been proposed that the principles of tensional integrity (tensegrity) may be a useful conceptual framework to explore and predict how molecules can function collectively as components of integrated, hierarchical systems, in the physical context of normal living cells, and tissues.[62]

If the information directing these processes are indeed "mechanogenomic" in nature, as described by Maniotis et al[2] in this issue of *The American Journal of Pathology*, the apparent complexity of development and cancer may be better understood by insight into how the genome is part of a larger machine that can interpret different signals only in a limited number of ways that is restricted by the ECM microenvironment, and the type of mechanogenomic architecture that environment erects within the cell and nucleus.

In a broader context, understanding of cell structure-gene expression interrelationships is in its infancy. However, there is accruing evidence for a central role of cellular microenvironments in the detection, integration, and execution of regulatory signals. Support for focal thresholds of genes, transcripts, receptors, and regulatory factors that optimize informative encounters to support gene expression, replication, and repair at multiple levels has emerged from interrogation of fundamental parameters of biological control. Additional insight into the regulation of gene expression should be forthcoming from further exploration of the mechanisms and underlying parameters that mediate the multistep dynamic positioning and combinatorial association of architecturally organized regulatory macromolecules in the nucleus, cytoplasm, and extracellular matrix. Despite compelling support for the physiological relevance of intracellular regulatory domains, there is a requirement to define rate limiting parameters of mechanisms that mediate the temporal and spatial components of cell structure-gene expression interrelationships. The rules governing organization of the regulatory machinery in the three-dimensional context of cellular architecture are being functionally enhanced by the combined applications of molecular, cellular, and *in vivo* genetic approaches. A prominent position for mechanogenomic control is an important consideration. It is realistic to expect that mechanistic explanations for the dynamic organization, assembly, and activities of regulatory machinery in cellular microenvironments which are subtly or catastrophically compromised in many diseases, including cancer, can provide a platform for novel approaches to diagnosis and treatment. It is further necessary to incorporate consideration of mechanogenomic control into mechanisms that may be operative in physiological processes that include, but are not restricted to, cell motility that is coupled with development, differentiation, tissue turnover, and remodeling, as well as the common denominators that may link mechanogenomic components of control with tumor progression and metastasis.

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