Journal of Cellular Biochemistry 104:1964–1987 (2008)

Nuclear Microenvironments in Cancer Series

Mechanotransduction From the ECM to the Genome: Are the Pieces now in Place?

Randall S. Gieni and Michael J. Hendzel*

Cross Cancer Institute and Department of Oncology, Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 1Z2

Abstract A multitude of biochemical signaling processes have been characterized that affect gene expression and cellular activity. However, living cells often need to integrate biochemical signals with mechanical information from their microenvironment as they respond. In fact, the signals received by shape alone can dictate cell fate. This mechanotrasduction of information is powerful, eliciting proliferation, differentiation, or apoptosis in a manner dependent upon the extent of physical deformation. The cells internal ''prestressed'' structure and its ''hardwired'' interaction with the extra-cellular matrix (ECM) appear to confer this ability to filter biochemical signals and decide between divergent cell functions influenced by the nature of signals from the mechanical environment. In some instances mechanical signaling through the tissue microenvironment has been shown to be dominant over genomic defects, imparting a normal phenotype on cells that otherwise have transforming genetic lesions. This mechanical control of phenotype is postulated to have a central role in embryogenesis, tissue physiology as well as the pathology of a wide variety of diseases, including cancer. We will briefly review studies showing physical continuity between the external cellular microenvironment and the interior of the cell nucleus. Newly characterized structures, termed nuclear envelope lamina spanning complexes (NELSC), and their interactions will be described as part of a model for mechanical transduction of extracellular cues from the ECM to the genome. J. Cell. Biochem. 104: 1964–1987, 2008.

Key words: mechanotransduction; cell nucleus; chromatin

TENSEGRITY

Life is both physical and chemical. This is not disputed at most scales of organization. Remarkably, at the cellular scale, most phenomena are ascribed solely to chemical mechanisms. Nonetheless, there is direct experimental evidence for physical continuity between the extracellular matrix (ECM) and nucleus mediated through the cytoskeleton (CSK) [Bloom et al., 1996; Maniotis et al., 1997b; Maxwell and Hendzel, 2001]. Many studies show changes in cell behavior that correlate with or depend upon mechanical signals delivered to the cell via its interaction with the environment. These include studies showing cell fate to be dependent on cell shape [Folkman and Moscona, 1978; Ben-Ze'ev et al., 1980, 1988; Glowacki et al., 1983; Ingber and Folkman, 1989; Opas, 1989; Ingber, 1990; Mooney et al., 1992; Singhvi et al., 1994; Chen et al., 1997; Dike et al., 1999; Niland et al., 2001]. Studies showing differences in gene expression between cells with differing adhesion [Li et al., 1987; Yan et al., 2000; Balda and Matter, 2003; Dalby et al., 2005, 2007a] and studies showing differences in the phenotype and/or gene expression between cells cultured in ECM gels (3D) versus on flat culture plates (2D) [Li et al., 1987; Petersen et al., 1992; Weaver et al., 1996, 1997; Lelievre et al.,

This article contains supplementary material, which may be viewed at the Journal of Cellular Biochemistry website at http://www.interscience.wiley.com/jpages/0730-2312/suppmat/index.html.

Grant sponsor: Canadian Institutes of Health Research; Grant sponsor: National Cancer Institute of Canada.

*Correspondence to: Michael J. Hendzel, Cross Cancer Institute, 11560 University Ave. NW, Edmonton, Alta., Canada T6G 1Z2. E-mail: michaelh@cancerboard.ab.ca

Received 8 March 2007; Accepted 12 March 2007

DOI 10.1002/jcb.21364

1998] among others. These results are not easily explained by models of the cell or the nucleus that treat them as an agglomeration of independent moieties floating in a protein-rich medium.

To explain the observed continuity and transmission of mechanical signals, Ingber proposed a tensegrity model of cellular architecture. The concept of tensegrity and its hierarchal nature in biology has been extensively reviewed [Ingber et al., 1994; Ingber, 1998, 2003a]. Tensegrity or tensional integrity refers to the stability of structures based on a synergy between balanced continuous tension and non-continuous compression components. The geodesic dome is the classical example of a tensegrity structure. Mechanical stress is distributed evenly over the entire surface via cross-linked triangular elements known as struts. In its simplest form, the cell can be modeled as a tensegrity structure containing a series of rods interconnected by elastic bands. This model mimics basic cell behavior in that it takes on a spherical shape similar to cells in suspension when unattached, but spreads, flattens and transfers stress to a rigid substrate when adherent [Ingber and Jamieson, 1985; Ingber, 2003a,b, 2006a,b].

Living cells are similarly stabilized through the establishment of this balance between tension and compression. Actomyosin contractile filaments cause continuous internal tension in the living cytoskeleton which is distributed by cross-linked bundles of microfilaments (MF) and microtubules (MT). In suspended cells this compressive force is counterbalanced mostly by transient MT bending and buckling, resulting in a state of isometric tension or prestress within the cell against which all external forces are imposed. As cells adhere, some of this tension is distributed to the ECM through adhesion points and ECM flexibility [Chrzanowska-Wodnicka and Burridge, 1996; Eastwood et al., 1998]. In an adherent state, cells shift compressive force back and forth between ECM attachments and MT's altering ECM stiffness [Ingber, 2003a,b; Hu et al., 2004]. Together the mechanical interaction between cells and between each cell and its ECM provides the entire tissue with a level of prestess. It is within this microenvironment of biochemical and mechanical signaling that cells function in vivo.

CONNECTIVITY FROM THE ECM TO THE NUCLEUS

While the continuity between focal adhesions, where cells interact with the ECM, and the cytoskeleton is well established, the transmission of forces from the cytoskeleton to the genome is more of an unknown. For mechanotransduction to directly impact upon gene expression, it is necessary that the cell be capable of transmitting forces from the cell surface directly to the genome. In this review, we provide an overview of the cellular systems capable of transmitting force from the extracellular environment to the interior of the nucleus and ask the question: Is there evidence for continuity between the ECM, the cytoskeleton, and the interior of the nucleus where the genome is housed?

Early studies showed that adhesion molecules such as integrins or cadherins but not a bound metabolic receptor (low density lipoprotein receptor) or histocompatibility antigens could transfer external mechanical force (twisting magnetic beads) to the internal CSK [Schiro et al., 1991; Schmidt et al., 1993]. As predicted by the tensegrity cell model these same receptors were also able to transfer internal CSK tension to the ECM. This "hardwired" connection was confirmed in studies showing that a pull on an ECM receptor (integrins) but not a bound metabolic receptor caused immediate realignment of cytoskeleton filaments distorting the nucleus and redistributing and elongating the nucleoli along the axis of the applied force [Maniotis et al., 1997b]. Stress induced multimolecular realignment extended deep into the interior of the nucleus where individual nucleoli could be seen to distort. Notably, the nucleoli moved towards the source of the applied force while fluid flow would be expected to move away from the source of the applied force as the plasma membrane on either site of the site of applied force collapse towards each other. With integrins, this transmission of mechanical information is mediated via the actin cytoskeleton through links between integrins and actin-binding proteins (talin, paxillin' vinculin). The transmission of force from the cytoplasm into the nuclear interior can also be observed in timelapse experiments of living cells. The latter also highlight a second feature of the continuity between the nucleus and the cytoskeleton—its dynamics. It is not uncommon for the nucleus to

rotate relative to a more stationary cell body. Figure 3 shows a human neuroblastoma cell labeled with RhdCTP, which incorporates into chromatin during synthesis, and DIC bright-field images, where the cell body and nucleoli can be clearly visualized. It can be seen the nucleoli can undergo dramatic distortions that are reversible in nature.

EFFECTS OF CELL SHAPE ON FATE

Cell shape can be a powerful determinant of fate. Numerous studies have examined the role that interactions with the ECM has on cell function and differentiation [Folkman and Moscona, 1978; Ben-Ze'ev et al., 1980, 1988; Glowacki et al., 1983; Li et al., 1987; Ingber and Folkman, 1989; Opas, 1989; Ingber, 1990; Mooney et al., 1992; Singhvi et al., 1994; Chen et al., 1997; Dike et al., 1999; Niland et al., 2001]. These studies, carried out with multiple cell types, utilize changes in the density of immobilized ECM, changes in the flexibility of ECM gels or micro-fabrication of planar ECM "islands" with defined size and shape to show a direct relationship between cell adhesion and function. With few exceptions, cells allowed full contact with competent ECM form adhesion foci, spread and then proliferate, while cells grown in conditions where they are unable to form strong ECM attachment round up and undergo programmed cell death (apoptosis). Cells allowed moderate levels of adhesion or cultured on ECM with a mechanical stiffness similar to that of their natural tissue [Engler et al., 2004] undergo differentiation. The elegance of this approach is that the total surface area of ECM bound to the cell can be maintained while controlling the degree of cell spreading by concentrating the signal in a single island or spreading the same surface area of ECM out over multiple small islands. Under these conditions, the biochemical signaling in response to mitogens [Ingber, 1990] or the insoluble ECM molecules themselves [Yan et al., 2000] is similar while cell shape and spreading is specifically modulated. For example, one recent study utilized pluripotent human mesenchymal stem cells (hMSC) shown to differentiate into adipocytes (fat cells) or osteoblasts (bone cells) depending on the presence of differentiation factors and the degree of cell adherence allowed [McBeath et al., 2004]. In cultures with a mixture of factors promoting both lineages, cell shape directed commitment with rounded cells developing into adipocytes and adherent cells becoming osteoblasts. Differentiation factors favoring development of adipocytes were unable to direct such development in strongly adherent cells. Moreover, culture of the hMSC in adherent or non-adherent conditions before addition of soluble differentiation factors biased the cells towards bone or fat cell differentiation, respectively, suggesting cell shape affected stem cell commitment upstream of the effect of differentiation factors. Increasing cellular contraction experimentally by increasing RhoA activity in these stem cells strongly biased them toward bone formation, but only in conditions where they were adherent. Increasing tension in a suspended cell was ineffective indicating that the role of shape acts prior to the effect of RhoA.

One recent study has connected cell shape to epigenetic changes in cellular chromatin. When a gastic carcinoma cell line was analyzed in suspension, cells showed elevated histone H3 acetylation at lysine 9 relative to the same cells grown on fibronectin [Kim et al., 2005]. Since histone acetylation is thought to relax chromatin structure, one possible explanation is that the apparent downregulation of histone deacetylase activity in these cells is a compensatory mechanism for decondensation driven by cell tension. The contribution of tension to chromatin structure is consistent with apparent changes in chromatin organization when cells are treated with the drug latrunculin A. Latrunculin A binds to actin monomers and drives a net depolymerization of the actin cytosekeleton. Nuclei in cells treated with this drug have a much more irregular nuclear shape and the chromatin often appears to be less dispersed within the nucleoplasm (Fig. 1).

RhoA SIGNALLING AS AN ACTIVATOR OF THE CELLULAR CONTRACTION PATHWAY

The actin cytoskeleton is likely responsible for the transmission of tension to the surface of the nucleus. Tensional force within the actomyosin contractile system is regulated in large part by the degree of phosphorylation of myosin light chain (MLC), for which signal transduction pathways are known [Kamm and Stull, 2001; Pfitzer et al., 2001]. The activation state of RhoA, a member of a family of small GTP binding proteins, controls the development of focal adhesions and stress fibers in adherent

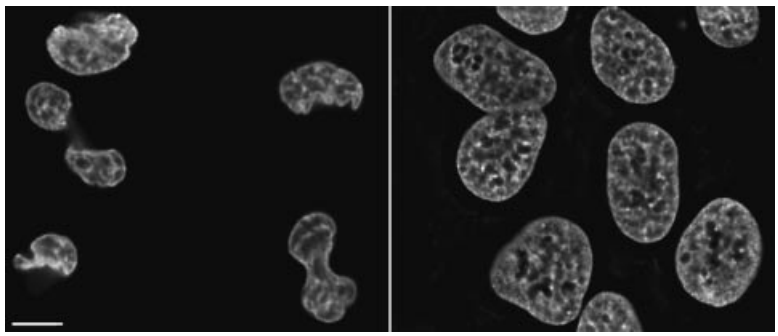

Fig. 1. Nuclear shape is dependent upon an intact actin cytoskeleton. HeLa cells were treated with latrunculin A for 60 min to depolymerize the actin cytoskeleton (**left panel**) or left untreated (**right panel**), fixed with paraformaldehyde, and then stained with the DNA binding dye, DAPI. The image shows a single optical section following deconvolution. The scale bar represents 10 μm.

fibroblasts [Chrzanowska-Wodnicka and Burridge, 1996]. RhoA's contractile functions include regulating cell ECM physical force interaction [Eastwood et al., 1998], cellular motility [Zhou and Kramer, 2005], and cytokensis [Matsumura, 2005]. RhoC has similar activity.

The activities of RhoA and RhoC on the actin cytoskeleton are mediated primarily through its downstream effectors ROCK [Bershadsky et al., 2006] and ROCK2 [Noma et al., 2006]. ROCK is a Rho-associated serine/threonine protein kinase which inactivates cofilin and myosin phosphatase to induce stabilization of filamentous actin and formation of stress fibers. Increases in internal cell tension are distributed through focal adhesions to the structure of the ECM, decreasing its flexibility [Eastwood et al., 1998]. The RhoA antagonist RhoE induces loss of stress fibers in cultured fibroblasts and epithelial cells in part by binding to RhoA-activated ROCK and inhibiting its activity. RhoE and its family members all relax cell tension and can themselves be regulated by phosphorylation [Riento et al., 2005]. Cell behavior, from motility [Kawada et al., 1999] to proliferation [Seasholtz et al., 1999; Iwamoto et al., 2000] are negatively affected by Y-27632 a non-specific ROCK inhibitor.

Artificial ECM gels showed that endogenous RhoA activity is minimal when cells are cultures on matrices engineered for flexibility but increases with increasing rigidity and that this effect is dependent on the actin cytoskeleton [Olson, 2004; Paszek et al., 2005]. Activation of RhoA by adherence to stiff ECM gels involves integrin clustering at focal adhesions and increased Erk activation. This invokes a mechanical positive feedback loop where cellular contraction increases internal cell tension forces which are then distributed to the ECM further increasing ECM gel rigidity. This feeds back positively to activate additional integrin clustering, Erk signaling and RhoA activation, continuing to enhance contraction. In this way, RhoA activation of the contractile pathway may stabilize the proliferative phenotype of cancer [Paszek et al., 2005]. This may be why cancer tissue is generally more rigid than its source tissue due to increased stiffness of the ECM [Paszek et al., 2005]. The back and forth interaction of the RhoA biochemical pathway and the physically integrated integrin/ECM/CSK pathway allows the cell to sense the physical properties of its surroundings [Yeung et al., 2005]. This crossregulation allows mechanical and biochemical information to merge providing dynamic two way sensing of the microenvironment. Indeed trophoblasts refuse to move onto areas coated with laminin 1 even though they have the physical means to do so. On some level they understand that the chemical properties of the laminin 1 patch are not conducive to their motile function [Klaffky et al., 2006].

If the regulation of cellular tension is important in cell transformation, maintenance, and/or progression in cancer, we would expect changes in the RhoA pathway to occur in human cancers. Consistent with this, RhoA over expression has been associated with colon, breast, lung, and testicular germ cell cancers and in head and neck squamous-cell carcinomas [Benitah et al., 2004]. This link to cancer progression is being vigorously investigated [Lin et al., 2007; Ogawa et al., 2007; Touge

et al., 2007; Xia and Land, 2007]. Because of its overall function in cell biology, RhoA is currently being investigated as a therapeutic target in cancer therapy [Fritz and Kaina, 2006].

ROLE OF TENSION IN EMBRYOGENESIS

If cell shape and tension are important for cell fate and function this mechanism would be operative during embryogenesis. One of the key early stages in all embryos is the transformation between blastomere and blastosphere. It is during this switch that cells secrete an endogenous ECM/basement membrane between adjacent layers. Through apopotosis, this becomes the blastosphere, the first ordered tissue, with cells on one side being trophoblast and the other embryoblast.

Much of the work in this area has focused on gene switches, however, one model explains embryonic bud formation in terms of changes in local tensional forces [Ingber and Jamieson, 1985; Huang and Ingber, 1999]. Because tissues are themselves pre-stressed by the dynamics of tension and compression, subtle degradation or unraveling within the ECM components allow the local basement membrane to stretch. While not affecting neighboring cells, this "run in a stocking" effect stretches those locally attached cells activating cell proliferation and bud formation. Cell stretch induced budding then repeats outwardly to produce the fractal-like pattern uniformly found in all tissues and species [Ingber, 2006b].

This model was tested experimentally by examining the effect of varying cellular tension within explanted whole lung organ rudiments on epithelial cell budding [Moore et al., 2005]. In cultures where cellular tension was diminished by the addition of the ROCK inhibitor Y27632 (or addition of other inhibitors of the contractile pathway) areas of locally thinning basement membrane were lost and epithelial branching minimized. Increasing cell tension by activating endogenous Rho with activator CNF-1 significantly increased bud formation. Angiogenesis (capillary elongation) also increased or decreased in direct correlation with enhanced or diminished cellular tension keeping the developing tissue well vascularized. In this way mechanotransduction of tensional information may dictate the order of developing tissues and help to build the body's hierarchal tensegrity structure.

A separate study examined this pathway using conditional expression of a dominant-negative form of RhoA or ROCK in transgenic mice to suppress the activity of these signaling molecules in early embryonic development. The dominant-negative mice had dramatically reduced motor neuron cell numbers caused by an increased rate of motor cell apoptosis in vivo [Kobayashi et al., 2004]. Another study found RhoA to be very active in embryos and that RhoA inhibition by RNAi caused severe lethal heart and head defects [Kaarbo et al., 2003].

TISSUE MICROENVIRONMENT CONTROL OVER GENOTYPE

We must remember that the cells in our body all share the same genes, yet these genes are expressed in a tissue specific manner to allow homeostatic function within. We have so far only examined experiments with cells grown in 2D cultures. In tissues, cells are adherent not only to the basement membrane but also to a surrounding ECM gel and to adjacent cells in an orientation somewhat similar to eggs in a carton. Many cell types also have the ability to alter their own local ECM/basement membrane makeup by secreting ECM components and growth factors, degrading ECM chemistry by enzyme secretion, and by applying physical force. It is in this context that focal adhesions between a cell and the surrounding ECM along with cell: cell contact constrains the cell but also provides a means to order cellular architecture. If integrins are "hardwired" to the nucleus through the CSK, this 3D configuration may also act to order the nucleus and perhaps coordinate transcription. In this respect, it is notable that most studies on genome organization have focused upon measuring chromosome territory organization or gene-specific locations in 2D tissue culture models. It is notable that cells with similar functions in different organs have similar shape whereas it is groups of specialized organ cells that have distinct shape, orientation, and function. Whether or not this confers specific changes in genome organization remains to be determined.

By controlling gene accessibility, the tissue's mechanical environment may define the cells response to biochemical signals. During tissue damage, disruption of this architecture would alter the cells responsiveness to biochemical

signaling and rapid internal remodeling of the hardwired pathway could bring formerly sequestered areas of chromatin containing genes essential for tissue repair both into a transcriptionally active conformation and into a nuclear compartment that is transcriptionally competent.

Epidemiological studies of cancer provide indirect evidence that the mechanical microenvironment itself may exert control over gene expression and thus phenotype. Studies of women exposed to toxic levels of X-rays from clinical therapy and from the atomic bombs used on Japan [Carmichael et al., 2003] or toxic X-ray fluoroscope exposure during screening for tuberculosis in the USA [Hrubec et al., 1989] showed higher incidence of cancer among those exposed as young women than was expected for the population at large. The tumors had a latency of 25–30 years, developing at a time at which the microenvironment of the irradiated tissue also lost structural normality. In some cases, changes in the organ microenvironment (stroma) occur before the onset of tumor formation and are thought to contribute to the genomic instability that destines a cell to become cancerous [Sternlicht et al., 1999]. Support for this interpretation comes from the finding that muscle stem cells differentiate into mature muscle when injected into normal skeletal muscle tissue but these same cells form tumors if the host skeletal muscle is first exposed to gamma irradiation [Morgan et al., 2002].

A similar result is seen with individuals carrying heritable cancer syndromes where each cell in the body carries the mutation yet tumors generally arise in a single tissue and only after sufficient latency [Nagy et al., 2004]. The same is true for engineered oncogene-expressing mice whose tumors are not universal but generally arise only from occasional cells and only after an appropriate latency period [Stewart et al., 1984]. The explanation for such a long latency between initial genetic damage and development of cancer has been that numerous deleterious genetic lesions must accumulate over time to allow phenotypic transformation and tumor progression. An alternative explanation would be that normal tissue architecture restrains expression of most deleterious genetic effects holding the cell in a state of homeostaisis via its mechanical connections to the tissue microenvironment. In this model natural tissue structure degradation with age eventually removes mechanical restraint allowing expression of the cancerous phenotype. These are not mutually exclusive ideas as controls exerted by the mechanical microenvironment could also be subverted by mutation over time.

If genes were in themselves autonomous, then cells isolated in culture should continue to function as in vivo. This is not the case. Isolated cells generally lose most functional differentiation in 2D culture. However, many tissue-specific traits can be remembered by culturing the cells in a microenvironment resembling the original tissue [Bissell, 1981]. Bissel has long argued for a role of the tissue microenvironment in the phenotypic control of genetically abnormal cells [Bissell, 1981; Bissell and Labarge, 2005; Bissell et al., 2005]. In these studies, cells are grown in 3D cultures of ECM (Matrigel) where the cells can migrate into the gel and setup ECM and cell to cell connections in a more physiological setting [Weaver et al., 1996]. It is known that cells can have a remarkable degree of genetic damage while remaining phenotypically dormant [Chin et al., 2004]. In the Bissel laboratory a luminal epithelial cell line (HMT-3522) isolated from a reduction mammoplasty was used to derive S1 cells that were found to have a number of mutations. When these cells were seeded in 3D cultures that resemble the in vivo tissue microenvironment, they differentiate to form structural mimics of true mammary acini found in vivo [Petersen et al., 1992]. Passage of S1 cells in the absence of EGF lead to the isolation of T4-2 cells that contain additional genetic lesions, form tumors in mice, and form non-polarized disorganized masses in 3D culture [Briand et al., 1987]. In this model S1 HMT-3522 cells are thought to represent genetically compromised cells predisposed to malignancy but which can still be directed to normal differentiation and homeostasis by interactions with a physiological microenvironment. In contrast, the T4-2 cells are thought to represent a state where genetic damage is sufficient to bypass this regulation.

When comparing the cell lines, T4-2 was found to have altered biochemical signal pathways including deregulated expression and signaling through beta-1 integrins. Blocking these pathways with antibodies or pharmacological reagents returned environmental control over phenotype in 3D cultures, allowing normal

acini-like structures to form from genetically tumerogenic cells [Wang et al., 1998, 2002a]. Importantly, reducing beta-1 integrin levels in T4-2 to the that found in S1 cells with anti-integrin antibody completely reverted T4-2 to a normal phenotype allowing acini formation in 3D culture [Weaver et al., 1997]. This finding in particular is suggestive of a role for mechanically transduced signals in the reversion process. The group has postulated a link between the extracellular microenvironment and gene expression similar to the "hardwired" explanation of Ingber [Lelievre et al., 1996].

CHANGES IN NUCLEAR ORGANIZATION IN 2D VERSUS 3D CULTURE SYSTEMS

There have been very few studies comparing nuclear organization in 2D versus 3D. Culture systems of HMT-3522 cells showed qualitative differences in the distribution of the structural proteins NuMA and lamin B, as well as the splicing factor SRm160 and the tumor suppressor/cell cycle regulator protein Rb. A potential role for NuMA in nuclear organization or gene regulation is suggested by the observations that its pattern of nuclear distribution depends upon cell phenotype and that it interacts and/or colocalizes with transcription factors [Lelievre et al., 1998]. This role was supported by the finding that disruption of NuMA by antibody in vivo resulted in the loss of acinar differentiation.

Further study showed that expression of a portion of the C terminus of NuMA also inhibited acinar differentiation and caused the redistribution of NuMA, the euchromatin marker acetylated histone H4, the heterochromatin marker trimethylated lysine 20 of histone H4, and regions of deoxyribonuclease I-sensitive chromatin [Abad et al., 2007]. Using confocal microscopy and a novel analysis technique, termed automated local bright feature image analysis, to examine the distribution of fluorescently stained NuMA in large numbers of nuclei from S1 cells undergoing acinar differentiation, the study was able to quantify differences in NuMA staining between experimental groups [Knowles et al., 2006]. The results revealed marked changes in the distribution of the density of NuMA bright features when S1 cells underwent phenotypically normal acinar morphogenesis in 3D cultures. When T4-2 cells were 3D cultured and allowed to form irregular tumor masses no reorganization of NuMA was apparent. These findings suggest that 3D culture of cells caused a reorganization of NuMA and that this change affected mammary epithelial differentiation by influencing the organization of chromatin. The mechanism(s) responsible for these changes in NuMA distribution require further characterization to determine if this pathway is widely important in tissue differentiation.

This body of work not only suggests that the tissue microenvironment (stroma) controls the phenotype of cells, but that an altered microenvironment contributes to, or can even be the cause of, genetic instability required for cancer development. In support of this, some cases where a genetic defect increases the incidence of cancer are now shown to act through changes in gene expression in the stroma [Jacoby et al., 1997; Howe et al., 1998]. Disruption of tissue structure itself may be an oncogenic event even in the absence of initial genetic mutation [Sternlicht et al., 1999; Maffini et al., 2004; Bissell and Labarge, 2005; Bissell et al., 2005]. One such pathway may be the aberrant secretion of matrix metalloproteinase (MMP) by stromal cells. This enzyme causes degradation of the ECM, and its overexpression has been shown to thin the ECM and alter cell shape in vitro [Bissell, 2007], and increase the incidence of tumor formation in vivo [Sternlicht et al., 1999]. Recent findings support the hypothesis that stromal abnormality can affect cancer induction or progression [de Yzaguirre et al., 2006; Infante et al., 2007; Rodriguez-Canales et al., 2007]. Normal aging leads to disruption of tissue and ECM architecture and this may have a similar destabilizing effect on cancer prone cells.

ECM-dependent chromatin organization was also examined by *Alu*I restriction enzyme sensitivity. *Alu1* is a restriction enzyme with a recognition site that is found within the repetitive Alu sequences within the human genome. It was observed that normal cells were relatively more exposed compared to their malignant counterparts in cultured cells and human tumor samples [Maniotis et al., 2005]. Somewhat unexpected, however, was the finding the *Alu1* sensitivity in each cell type was also regulated by cell shape. Chromatin in cells cultured in conditions of maximal adherence (with laminin or 3D culture) was poorly accessible to *Alu*1 and became much more sensitive

when grown in serum containing medium or when cultured on collagen. Disruption of the actin cytoskeleton led to exposure, while disruption of MT or IF inhibited digestion. The *Alu1* sensitivity of DNA in all the cells tested was exquisitely regulated by the mechanical microenvironment demonstrating that cell adhesion promotes *Alu1*-resistant chromatin order irrespective of cell type [Maniotis et al., 2005].

HOW ARE MECHANICAL CUES TRANSMITTED FROM THE CSK TO THE NUCLEUS?

The nuclear envelope consists of an outer nuclear membrane (ONM), which is continuous with the rough endoplasmic reticulum membrane, separated by an approximate 50 nm perinuclear space (PNS) from an inner nuclear membrane (INM) is continuous with the outer membrane at nuclear pore complexes [Gerace and Burke, 1988]. Given evidence that changes in cell shape and cell–ECM interactions can alter gene expression, it is important to understand whether there are mechanisms to transmit mechanical signals from the cell surface or cytoplasm to the cell nucleus. Characterization of the coupling between KASH-domain and SUN-domain integral nuclear membrane proteins reveal an answer. These proteins interact across the nuclear envelope (NE) providing a functional and mechanical link between the CSK and nucleus [Starr and Han, 2003]. The inner membrane is supported by the associated nuclear lamina, a mesh like network of type IV intermediate filament proteins composed of lamins and lamin associated proteins (Table I) that has many of the characteristics required for mechanical signal transduction (discussed below).

Nesprins are a family of proteins encoded by three genes in humans, two of which produce at least 12 isoforms generated by alternative transcription initiation and termination or alternative mRNA splicing [Starr and Fischer, 2005; Warren et al., 2005]. Some nesprins are massive (>1 MDa) and are thought to be oriented in the outer nuclear membrane (ONM) such that they extend up to 500 nm into the cytoplasm. The large variants have a conserved C-terminal KASH domain with a single transmembrane segment linked by a variable number of spectrin-repeat rod domains to an N-terminal paired, actin-binding, calponin-homology domain [Zhang et al., 2001, 2005]. The giant isoforms of Nesprin-1 and

TABLE I. Nuclear Lamina Associated Proteins and Functions

Properties	Name	Activities both known and hypothetical(?)
LEM domain proteins	MAN1	Binds BAF. Associates with emerin in vivo. Along with emerin is required for cell division and chromosome segregation in *C. elegans*. Chromatin condensation. Mutation associated with laminopathy like bone abnormalities. Tissue specific gene expression?
	LEM2	Binds BAF. Involved in the integrity of the nuclear envelope and nuclear organization. Cell viability. Tissue specific gene expression?
	LAP2	Binds BAF. Isoforms have tissue specific expression patterns. Mitotic chromosome segregation. Mutation associated with cardiac laminopathy. Tissue specific gene expression?
Emerin associated proteins	BAF	Chromatin remodeling, transcriptional regulation. Tissue specific gene expression by binding BAF like regulator?
	GCL1	Chromatin condensation. Nuclear chromatin structure stabilization. Transmission of force through nucleus?
	Actin	Potential nuclear actin cortical network? Structural arrangement of nucleus. Transcription complex formation. Active transport? Transmission of force through nucleus?
	Myosin	Required for transcription? Active transport mechanism(s)? Interactions with actin for active transport? Nuclear tension control? Force generation in nucleus?
	BtF	Apoptosis initiation. Sequestered by binding emerin. Regulation of cell cycle, cell death?
	YT521-B	mRNA splicing factor. Splice site dependent on emerin binding. Tissue specific isoforms?
Lamina associated proteins	LAP1	Kinase activity. Interphase chromatin organization? Affects emerin activity?
	Lamina B receptor	Direct chromatin binding and additional interactions via HP1. Chromatin condensation and stability at nuclear envelope. Transmission of force through nucleus?
	HP1	Chromatin organization at nuclear envelope. Heterochromatin formation.
	Titin	Nuclear organization. Structural nuclear component. Kinase activity. Chromosome packaging. Transmission of force through nucleus?

Nesprin-2 are restricted by size to the ONM where they interact in the PNS with Sun-domain proteins. Smaller isoforms including Nesprin-1α [Mislow et al., 2002] and some Nesprin-2 isoforms [Zhang et al., 2005] are associated with the INM and bind to the nuclear lamina and the actin and BAF binding protein emerin (discussed below, Table I). A subset of smaller Nesprin-2 proteins containing the calponin-homology domain are also found in the nuclear interior bound in heterochromatin complexes in close association with the nuclear lamina [Zhang et al., 2001, 2005]. The role of these interactions remains to be determined.

The SUN domain was characterized as a roughly 120 amino acid domain in the C-terminus of *Caenorhabditis elegans* UNC-84 protein, which is found as an integral protein of the INM [Malone et al., 1999]. Humans have at least four SUN domain genes, SUN1, SUN2, SUN3, and SPAG4 [Starr and Fischer, 2005]. SUN1 and SUN2 proteins are found on the INM oriented so that residues within and outside the SUN domain interact with the KASH domain of giant Nesprins across the PNS [Padmakumar et al., 2005]. This interaction gives stability to the PNS which widens when the SUN/KASH domain interactions are disrupted [Crisp et al., 2006]. The human SUN1 transmembrane portion spans the INM three times suggesting that it anchors mechanical load bearing structures to the membrane. SUN proteins form homodimers, and perhaps heterodimers [Tzur et al., 2006], allowing each dimer to bind two KASH proteins. This configuration allows the SUN/KASH bridge to physically link the nucleus to the actin cytoskeleton.

THE LINC COMPLEX COUPLES THE NUCLEUS TO THE ECM AND CYTOSKELETON

The LINC complex (*li*nks the *n*ucleskeleton and *c*ytoskeleton) is an experimentally characterized arrangement where INM SUN1 or SUN2 dimers bind the KASH domain of an ONM giant Nesprin2 [Crisp et al., 2006]. Another potential arrangement of INM SUN1 or SUN2 dimers is a case where one SUN domain binds the KASH domain of an ONM bound giant Nesprin, while the other SUN domain interacts with the KASH domain of an INM integral Nesprin-1α. Nesprin-1α may be stabilized in its association with the LINC complex via its binding to both lamins and emerin [Mislow et al., 2002]. In this conformation the actin CSK would be linked by this complex to the nuclear lamina and, through the actin binding properties of emerin, to the ill defined polymeric form of actin found in the nucleus [Holaska et al., 2004; McDonald et al., 2006]. The nuclear lamina and its components are discussed in detail below as potential mediators of mechanical signal transduction.

The existence of the LINC complex brings the structure of the nucleus directly into the physical realm of the CSK. This structure allows physical interaction between the nucleus, the CSK, ECM, and cell–cell adhesion complexes allowing the possibility of physical signaling from the ECM to the nucleus. We speculate that this tensegrity pathway may help regulate homeostasis and allow remodeling of gene expression profiles to reflect changes in the mechanical microenvironment. This is consistent with the cell behaving as a tensegrity structure and provides a mechanism for changes in the stroma to contribute to cell transformation.

Nesprin-3 has been characterized in humans and is localized to the ONM. Nesprin-3 lacks the calponin-homology domain but contains a region that binds plectin, a protein which associates with intermediate filaments (IF) and can directly cross-link them with actin [Wilhelmsen et al., 2005]. Although the interaction that secures Nesprin-3 at the ONM is not characterized, structural similarity with Nesprin-1 in the SUN binding regions suggests that interaction with SUN dimers may also anchor Nesprin-3. This interaction with the IF system may physically link the nucleus with hemidesmosomes and IF associated cell surface complexes including the integrins [Wilhelmsen et al., 2005]. This nuclear envelope bridge may explain why, when cardiac myocytes are stretched, there are changes in the orientation of the intermediate filament network connecting desmosomes with the nuclear lamina and in the spatial arrangement of chromatin associated with the nuclear envelope. This chromatin rearrangement is thought to be involved in the mechanical activation of hypertrophy-associated genes [Bloom et al., 1996].

The model organism *C. elegans* has revealed some functions of these SUN/KASH interactions. The *C. elegans* UNC-84 protein is a SUN domain protein [Malone et al., 1999] which as a

monomer is capable of binding the KASH domain proteins ANC-1 [Starr and Han, 2003] or UNC-83 [Starr et al., 2001]. Interaction with ANC-1 physically links the nucleus to the actin CSK while interaction with UNC-83 attaches the nucleus with MTs through an undefined motor protein. In *C. elegans*, disruption of either of these pathways affects nuclear positioning and migration in distinct tissues.

Matefin/SUN-1 is a SUN-domain protein of *C. elegans* that may form dimers and interacts with ZYG-12, a KASH domain protein of the ONM. ZYG-12 is able to bind the dynein subunit DLI– and is required for dynein localization to the nuclear envelope. It is proposed that dynein and ZYG-12 move the centrosome/microtubule organizing center (MTOC) toward the nucleus, followed by a ZYG-12/SUN-1 bridge dependent anchorage. In *C. elegans* the interaction of ZYG-12 with SUN-1 is necessary to attach the centrosomes to the nucleus during embryogenesis [Malone et al., 2003]. This SUN/KASH pair is expressed only in germ cell lines with SUN-1 present during embryogenesis being maternally derived. Embryos whose SUN-1 expression was inhibited by feeding mother worms bacteria expressing SUN-1 RNAi die at an early 300-cell embryo stage and display defective nuclear structure, DNA content, and chromatin morphology [Fridkin et al., 2004] suggesting a role for this bridging complex in *C. elegans* developmental gene expression.

The full range of interactions between KASH and SUN domain members across the NE remains to be elucidated and their roles determined experimentally. The experimental results to date indicate that SUN domain proteins that span the inner nuclear membrane interact in the perinuclear space with KASH domain proteins that span the outer nuclear envelope. The latter interact with components of the cytoskeleton and provide the molecular basis for physical continuity between the cytoskeleton and the nuclear interior. Thus, the LINC complex provides a pathway for the transduction of mechanical signals from the ECM to the genome.

THE NUCLEAR LAMINA AS A FORCE TRANSMITTING STRUCTURE

The nuclear lamina is a thin mesh-like structure intimately associated with the inner nuclear membrane. It is composed of a network of intermediate filament proteins including lamins A and C from the LMNA gene locus, and lamins B1 and B2 from LMNB [Capell and Collins, 2006]. Physically associated proteins include emerin [Manilal et al., 1998], MAN1 [Lin et al., 2000], LAP1 [Martin et al., 1995], LAP2 [Furukawa et al., 1995], LEM2 [Brachner et al., 2005], the lamin B receptor (LBR) [Worman et al., 1988], nuclear titin [Zastrow et al., 2006] and indirectly BAF [Shumaker et al., 2001; Mansharamani and Wilson, 2005].

The entire complex is of particular relevance to mechanotransduction because it forms a scaffold that binds LINC complex thereby connecting the nuclear surface to the CSK and to chromatin. The lamina affects chromatin structure by binding it directly at many attachment points on the inner surface [Zastrow et al., 2004] and through the effect of associated proteins that themselves bind chromatin. In fact, most chromosomes have direct contacts between heterochromatin domains and the nuclear lamina [Ferreira et al., 1997; Sadoni et al., 1999]. Since mechanical signal transduction would have to proceed through the nuclear lamina, it is a prime candidate to participate in signaling between the environment and the genome.

The direct attachment points between lamins and chromatin and those provided by associated proteins [Lee et al., 2001; Morris, 2001; Ostlund and Worman, 2003; Zastrow et al., 2004] may participate in heterochromatin formation at the nuclear periphery. The fact that chromatin close to the nuclear lamina, the perinuclear heterochromatin, is transcriptionally silent and that spatial positioning and heterochromatin formation have roles in transcriptional regulation [Brown et al., 1997; Kosak et al., 2002; Zink et al., 2004; Goldmit et al., 2005; Parker et al., 2005; Wegel and Shaw, 2005; Zardo et al., 2005; Moss and Wallrath, 2007] provide evidence that the nuclear lamina may transcriptionally repress transcription.

Interestingly, alterations of cell and nuclear shape in cancer cells are often associated with loss of heterochromatin order [Cremer et al., 2003; Zink et al., 2004; Prokocimer et al., 2006]. It is possible that alterations in the attachment of cancer cells with the ECM affect nuclear shape and NELSC-mediated heterochromatin formation such that gene expression profiles are altered, contributing to disease progression.

NUCLEAR ENVELOPE-LAMINA SPANNING COMPLEXES (NELSC) AND THE CONTINUITY OF THE CYTOSKELETON WITH CHROMATIN

A full discussion of lamina associated proteins and their actions are beyond the scope of this review. Some associated proteins are summarized in Table I. Of particular relevance are the emerin related LEM domain containing integral INM proteins MAN1 which co-localizes with emerin at the NE [Lin et al., 2000; Liu et al., 2003; Mansharamani and Wilson, 2005], LAP2 [Shumaker et al., 2001, #91; Schoft et al., 2003, #266; Taylor et al., 2005, #130] and LEM2 [Brachner et al., 2005, #95; Ulbert et al., 2006, #119]. BAF (barrier to autointegration) binds the LEM domain in vivo and has chromatin remodeling activities and influences transcription [Segura-Totten et al., 2002; Zhao et al., 2005]. GCL1 (germ cell less-1) also binds the LEM domain with lower affinity and is required for nuclear integrity and proper chromatin condensation [Kimura et al., 2003]. LAP1 is an integral protein of the INM that forms multimeric assemblies with protein kinase activity which is suspended in the inner nuclear membrane and are specifically associated with B-type lamins [Maison et al., 1997, #254]. LBR (lamin B receptor) is a non-LEM containing protein that binds lamin B and affects chromatin dynamics through binding HP1 [Ye et al., 1997] which is thought to provide structural integrity to bound chromatin domains at the nuclear envelope [Makatsori et al., 2004; Worman and Courvalin, 2005]. The huge structural molecule titin also binds to the nuclear lamina. It has a protein kinase domain and may provide a structural component to the complex [Zastrow et al., 2006]. A number of proteins are lamina associated by physically binding to emerin. These include BtF, a molecule sequestered by emerin that in its free form is necessary for the induction of the apoptosis cell death pathway [Haraguchi et al., 2004]. YT521-B is a RNA splicing factor whose splicing site selection is influenced by its association with emerin [Wilkinson et al., 2003]. Other structural interactions with emerin include its binding of both actin [Holaska et al., 2004] and nuclear myosin [Bengtsson and Wilson, 2004; Wilson et al., 2005], a combination capable of active movement through a myosin actin sliding mechanism. Other molecules associated with LEM domain proteins likely await characterization. Of the LEM domain proteins, only muscular dystrophy-related emerin has been well studied. The other LEM proteins may have variable activities based on their structure and potential to bind as yet unidentified factors. The combination of structural, transcriptional, apoptosis regulating and splicing factor associations make the nuclear lamina and associated protein complex capable of influencing many aspects of nuclear function.

Overexpression of the lamin binding domain of titin disrupted nuclear lamina integrity and produced nuclei that were herniated and misshapen [Zastrow et al., 2006]. Titins are also involved in chromosome condensation and chromosome segregation during mitosis in *Drosophila melanogaster* embryos [Machado and Andrew, 2000]. Titin bound to the nuclear lamina may give order to chromatin in the nucleus. The large size and chromatin binding potential of titin make it a candidate for altering interphase nuclear organization in response to mechanically transduced signals from the environment.

LEM domain nuclear lamina associated proteins Emerin, LAP2, MAN1, and LEM2 are also of particular interest to this discussion. The LEM domain of each protein binds BAF (barrier-to-autointegration factor) a conserved chromatin protein essential for cell viability named as a host factor for retroviral integration [Cai et al., 1998, 2001; Shumaker et al., 2001]. BAF cross-links DNA, has structural roles during nuclear assembly and dictates higher-order chromatin structure through undefined mechanisms [Chi, 2004; Segura-Totten and Wilson, 2004; Zhao et al., 2005]. BAF also represses gene expression by inhibiting transcriptional activators [Wang et al., 2002b]. Through their binding of BAF and interaction with chromatin, LEM proteins are thought to influence chromatin dynamics [Haraguchi et al., 2001; Segura-Totten et al., 2002; Liu et al., 2003; Chi, 2004; Dechat et al., 2004; Segura-Totten and Wilson, 2004; Shimi et al., 2004]. Three additional LEM domain proteins have been identified in humans [Lee and Wilson, 2004].

Emerin may be a key player in NELSC. In one LINC arrangement, SUN dimers bind an ONM giant Nesprin to an INM integral Nesprin-1α protein. Nesprin-1 α forms homodimers and binds directly to lamin A and emerin [Mislow et al., 2002]. The affinity of Nesprin-1α for

emerin is extremely high (4 nM) suggesting that Nesprin-1α might help anchor a molecular complex spanning the nuclear membrane [Tzur et al., 2006]. Such a bridging structure may be important in mechanical transduction based on the observation that emerin is an actin binding protein acting to accelerate actin filament formation [Holaska et al., 2004]. Lamin A also binds actin perhaps stabilizing this link [Shumaker et al., 2001]. Together the LINC complex and emerin bring the cytoplasmic actin and the uncharacterized nuclear actin polymer pathways together. Recent evidence of polymeric actins [McDonald et al., 2006] and myosin [Philimonenko et al., 2004; Kysela et al., 2005; Kahle et al., 2007] in the nucleus raise the possibility of active application of tension within the nucleus and directly upon chromatin. It is interesting to note that when actin is depolymerized, chromatin often appears more condensed and enriched on the lamina surface and the surface of the nucleolus (Fig. 1). Similarly, when the LINC complex is disrupted, chromatin is also more condensed (see Crisp et al., 2006 Figures 6 and 7; B. Burke and J.B. Rattner, personal communication). These observations are consistent with a role for actin-dependent force application playing an active role in decondensing chromatin.

ARE NUCLEAR ENVELOPE LAMINA SPANNING COMPLEXES (NELSC) NECESSARY?

There are no known survivable mutations of the B lamins in humans, presumably due to their critical activity during embryogenesis. Inhibiting lamin B expression in cultured mammalian cells with small interfering RNAs show lamins B1 and B2 to be essential for cell growth and viability [Harborth et al., 2001]. Genetically modified mice without expression of wild-type lamin B1 have bone and lung abnormalities during development and die shortly after birth [Vergnes et al., 2004].

Lmna knockout mice having only B lamins survive but grow slowly and die by 5 weeks of age with bone and muscle defects [Fong et al., 2006]. *Lmna*-null mouse embryo fibroblasts have grossly misshapen cell nuclei, decreased viability and impaired mechanically activated gene transcription [Lammerding et al., 2004, 2005]. In related studies, these same cells exhibited reduced mechanical stiffness linked to changes in CSK organization [Broers et al., 2004].

Inhibiting LEM2 expression in human cell lines severely diminishes cell viability with increased nuclear fragility [Ulbert et al., 2006]. In *C. elegans* Ce-emerin and Ce-MAN might have overlapping functions, as RNA interference-mediated knockdown of both proteins is lethal at the 100-cell embryonic stage whereas single knockdown of detectible Ce-emerin has no detectible phenotype while a 90% knockdown of Ce-MAN resulted in only 15% embryonic lethality [Liu et al., 2003]. Together the studies show an essential role in the embryo for LEM proteins and a significant crossover in their activity. The simplified system of *C. elegans* provides a look at an early evolutionary take on LEM biology. Perhaps the functions emerin has acquired since then are those responsible for the clinical manifestation of emerin mutation as disease today.

In humans, mutations in the LMNA gene encoding lamins A and C lead to a wide range of serious disorders collectively termed the laminopathies. The tissues most affected include muscle, tendon and bone resulting in muscular dystrophies, premature aging (progeria) syndrome, and cardiomyopathy [Capell and Collins, 2006; Parnaik and Manju, 2006]. The list of gene defects clinically manifested as laminopathies are diverse including rare lethal skin thinning syndromes (restrictive dermopathy) [Levy et al., 2005; Moulson et al., 2005], metabolic disorders of fat accumulation and loss associated with wasting [Agarwal and Garg, 2006], and nervous system cell abnormalities [Vital et al., 2005]. In addition to disease-causing mutations in the *LMNA* gene, mutations in genes encoding emerin [Emery, 1987; Bione et al., 1994; Manilal et al., 1996] and MAN1 [Hellemans et al., 2004] give rise to muscular dystrophy and bone-related diseases, respectively. Mutations in the lamin B receptor (LBR), and LAP2 can also result in laminopathy [Taylor et al., 2005; Worman and Courvalin, 2005].

Emerin is mislocalized in the endoplasmic reticulum in cells carrying some disease-linked mutations in *LMNA* or in cells that lack lamin A [Sullivan et al., 1999; Ostlund et al., 2001; Raharjo et al., 2001; Vaughan et al., 2001; Holt et al., 2003; Muchir et al., 2004], suggesting that interaction with lamin A is critical to retain

emerin at the nuclear periphery. In contrast lamin C-only mice have normal emerin distribution [Fong et al., 2006]. The toxic properties of accumulating pre-lamin A both disrupt emerin location and nuclear stability in the mutation leading to Hutchinson-Gilford progeria syndrome (HGPS). Pre-lamin A accumulation appears to be a common pathway through which nuclear fragility occurs in many LMNA mutations not yielding functional lamin A [Ostlund et al., 2001; Ostlund and Worman, 2003; Goldman et al., 2004; Capell and Collins, 2006; Young et al., 2006].

Whether clinical outcome is caused by gene regulation defects or by the structural defects and fragility seen in the nucleus of patients suffering these diseases remains debatable. These are not mutually exclusive notions and it is likely that both mechanisms contribute. Cells expressing many of these mutations have frail nuclei, low viability and display abnormal mechanical signal transduction [Fong et al., 2006]. Yet emerin deficient fibroblasts have normal nuclear integrity and viability but are still deficient in mechanically induced signaling [Lammerding et al., 2005]. Experiments comparing gene expression in fibroblasts from X-linked Emery-Dreifuss muscular dystrophy (X-EDMD) patients and controls show at least 60 affected genes, the expression of most being increased. Normal expression was restored for 28 of these genes by expressing wild-type emerin in the X-EDMD cells [Tsukahara et al., 2002]. In support of the hypothesis that NELCS are important for heterochromatin formation and sequestering genes in the nuclear periphery, mouse fibroblasts lacking A-type lamins and fibroblasts from HGPS (progeria) patients both exhibit a loss of heterochromatin at the nuclear lamina [Sullivan et al., 1999; Goldman et al., 2004; Nikolova et al., 2004]. It is likely that disruption of NELSC by disease causing mutations results in much of the clinical pathology. These rare but serious illnesses underscore the importance of an intact nuclear lamina in maintaining tissue physiology [Jacob and Garg, 2006]. The wide range of phenotypes expressed by defects in these molecules underscores their important role in diverse tissues.

CHROMATIN AND DIRECT MECHANOTRANSDUCTION

In order for mechanical cues to meaningfully regulate gene expression, the nucleus must demonstrate order. It is well established each chromosome maintains its own discrete territory during interphase in agreement with the topographical model of the nucleus [Manuelidis, 1985; Cremer et al., 1988, 2001; Kurz et al., 1996; Scheuermann et al., 2004; Bolzer et al., 2005; Foster and Bridger, 2005; Foster et al., 2005; Murmann et al., 2005; Shopland et al., 2006]. Several studies have shown that genes tend to be nonrandomly positioned and preferentially near the surface of chromosome territories [Kurz et al., 1996; Belmont et al., 1999; Volpi et al., 2000; Cremer et al., 2004]. Specific genes or chromosome regions that have been shown to be spatially organized within interphase nuclei include the major histocompatibility complex on chromosome 6 [Volpi et al., 2000], the epidermal differentiation complex on chromosome 1 [Williams et al., 2002] and the conserved 4.3 Mb 4 gene cluster section of mouse chromosome 14 [Shopland et al., 2006]. For a comprehensive review of the current knowledge on the organization of the nucleus see [O'Brien et al., 2003; Foster and Bridger, 2005; Bartova and Kozubek, 2006; Prokocimer et al., 2006]. The tensegrity model predicts that the three-dimensional organization of genes in chromosomal territories may allow regulation of their expression through physical interaction with force-transmitting structures within the nucleus, such as NELSC. Human endothelial cell genomes act as continuous, elastic structures [Maniotis et al., 1997a,b] consistent with the hypothesis that chromatin can respond to applied force by reversible decondensation.

The question thus arises whether or not gene expression can be altered through mechanically induced changes. A series of reports by Dalby et al. support this model. They examined changes in genome organization and gene expression in response to changes in the level of cellular adhesion. Using nanotopography they found fibroblast adhesion was marginally inhibited on lithographed surfaces and severely retarded (cells almost round) on hexagonal pitted surfaces. They show that fully attached cells have well rounded nuclei and a more diffuse and expanded nuclear lamina, that shrinks and becomes more dense as adhesion is diminished [Dalby, 2005; Dalby et al., 2007a]. Using microarray analysis it was demonstrated that modulating adhesion altered gene expression in fibroblasts in a stepwise manner accord-

ing to adhesive state [Dalby et al., 2005]. Altering cell adhesion by cultures on lithographed or pitted surfaces showed that as cell spreading was changed, so were the relative positions of chromosomes. The gene expression studies showed that the more adhesion was reduced, the further gene expression was reduced [Dalby et al., 2005, 2007b]. Changing adhesion reduced CSK organization, causing the nucleus and nuclear lamina to shrink. As the NELSC shrank the inter-centromere distances were reduced demonstrating that alteration of cell shape does indeed constrain the nucleus altering the relative conformation of chromosomal territories [Dalby et al., 2007a,b].

A MODEL FOR THE MECHANICAL TRANSDUCTION TO THE GENOME

Thus far, we have explained how forces can be transduced from the cell surface to the NELSC and the chromatin that associates with the nuclear lamina. If chromosomes are tethered solely to the nuclear lamina, the only opportunity for mechanotransduction to the genome comes with expansion of the nuclear lamina, which could modestly decondense the chromatin that is directly associated with the lamina through increasing the distance between the attachment sites. To transduce mechanical information throughout the genome and to target this information, it is necessary to have intranuclear chromatin attachments. The most effective means of transducing this information would be through a nuclear equivalent to the cytoskeleton, a karyoskeleton. The nuclear matrix, a network of proteins revealed after the removal of chromatin from isolated nuclei, is one such putative structure. The finding that in interphase cells, the nuclear matrix appears to interconnect different nuclear components, such as nucleoli, to each other and the surrounding cytoskeleton [Fey et al., 1984] suggests that such a network exists. This type of network was further visualized without the necessity for harsh preparation conditions by employing energy filtered transmission electron microscopy, where the chromatin, RNA, and protein network can be resolved based upon their composition [Hendzel et al., 1999] (Fig. 2). More recently, we have demonstrated that actin can be found in polymeric form in the nucleus [McDonald et al., 2006] and provided evidence that polymeric actin contributes to the insolubility during nuclear extraction that is used to operationally define nuclear matrix proteins [Andrin and Hendzel, 2004]. The coordinated movement of otherwise apparently independent structures in timelapse experiments provides further support that physical continuity extends deep into the interior of the nucleus (Figs. 3 and 4, Movies 1 and 2) as originally demonstrated by Maniotis et al. [1997b]. Nonetheless, the existence of a karyoskeleton has

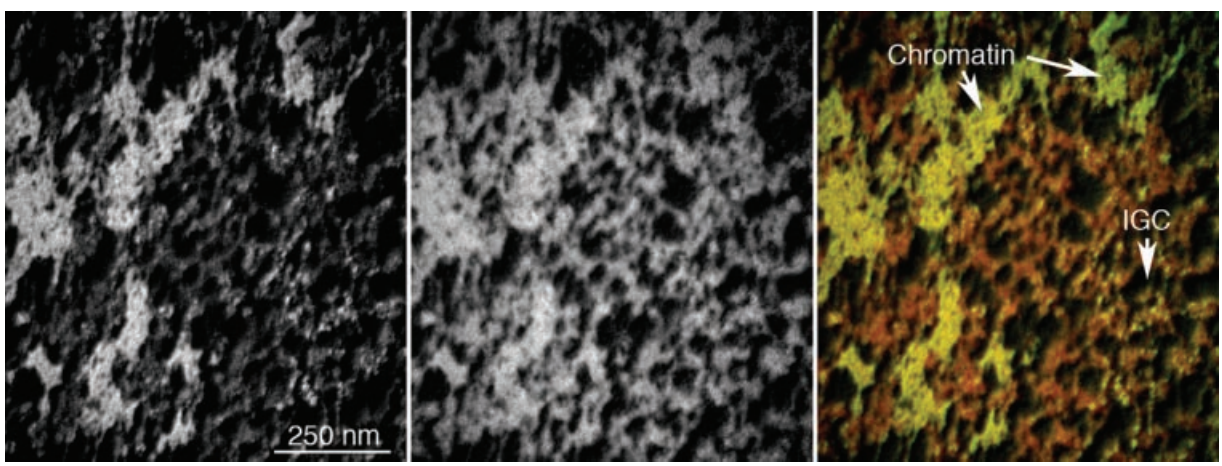

Fig. 2. Energy filtered transmission electron microscopy of the interphase nucleus. An approximately 40 nm thick section of a mouse 10T1/2 fibroblast cell was imaged by energy filtered transmission electron microscopy. The **left panel** shows a quantitative map of phosphorus, which primarily visualizes nucleic acids while the **center panel** shows a quantitative map of nitrogen, which visualizes both protein and nucleic acids. The **right panel** shows a composite of the two images with the phosphorus image false-colored green and the nitrogen image false-colored red. Chromatin and ribonucleoproteins appear yellow in the merged image while protein appears red-orange. Chromatin and an interchromatin granule cluster (IGC) (splicing factor compartment) are indicated with arrows.

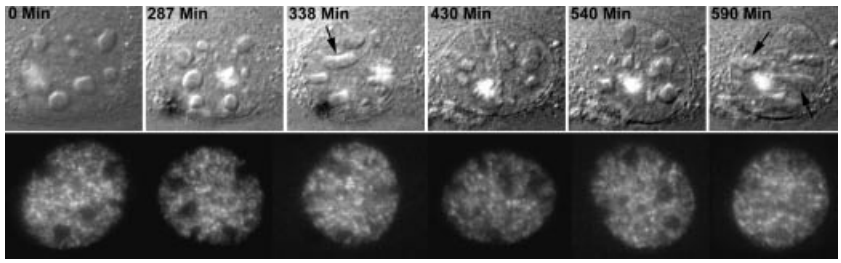

Fig. 3. Timelapse microscopy of a 10T1/2 cell nucleus. A mouse fibroblast cell was labeled with rhodamine dUTP (**bottom panels**) and 2D images were collected the following day by DIC (**top panel**) and fluorescence (bottom panel) microscopy across time. Over the time course, the nucleus is seen to rotate relative to the surrounding cytoplasm. The arrows indicate the positions of nucleoli which are distorted and then return to their original shape over time.

been controversial [Pederson, 2000]. Consequently, we have previously proposed that interactions between euchromatin and splicing factor compartments could provide the resistance (load) necessary to transform applied forces into changes in chromatin structure [Maxwell and Hendzel, 2001].

Two hypothetical models for the transmission of mechanical signals to the genome are presented in Figure 5. In Figure 5A, mechanotransduction via the LINC complex transfers tension generated in the cytoskeleton to chromosomes through the NELSC, which interacts with both chromatin associated with the nuclear lamina and the karyoskeleton. In Figure 5B, the LINC and NELSC are also responsible for the transmission of force to the nuclear interior. In this instance, however, there is no transmission of this force into the interior of the nucleus via a karyoskeleton. Rather, the force is transmitted through chromatin. Where chromatin interacts with large and relatively immobile nuclear structures, particularly splicing factor compartments (which are both large and are likely to have interactions with all chromosomes) a resistance to the applied force exists that may be sufficient to promote decondensation of the chromatin. In both cases, it is assumed that the chromatin, or, more likely, specific regions of chromosomes, are more likely to decondense than to remain stable enough to move the mass (load) of the intranuclear structure. The models differ in the targeting of this force. In the first model, the force can be transmitted throughout the chromosome and the sites of interaction between the interphase chromosomes and the karyoskeleton determine the targeting. Thus, a prediction of this model is that mechanically sensitive regions of the genome have regulated

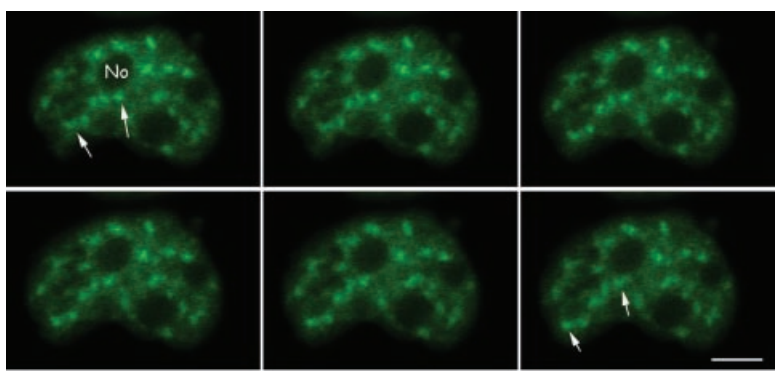

Fig. 4. Timelapse microscopy of a HeLa cell transfected with ASF-GFP. A HeLa cell was transfected with ASF-GFP, which enriches in splicing factor compartments, and imaged over time. The total elapsed time is 30 s (5 s intervals left to right then top to bottom). The arrow indicates a ''sting'' of five splicing factor compartments that synchronously move, across time, in unison with an extension of the nuclear envelope. Note the migration of these splicing factor compartments relative to the nucleolus positioned immediately above them. The scale bar represents 5 μm.

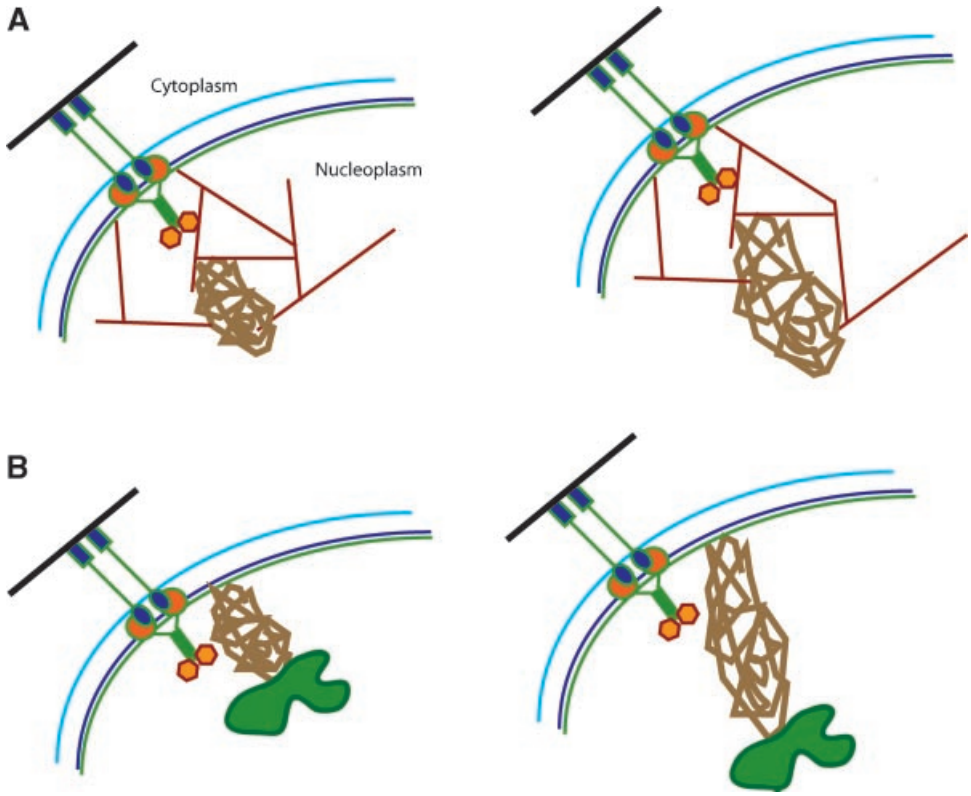

Fig. 5. Models for mechanotransduction from the cytoskeleton to the genome. The LINC complex is shown as a pair of Nesprin proteins (blue) interacting in the perinuclear space with a dimer of SUN protein (orange). In (**A**) the chromatin (brown) is connected to the nuclear lamina (green curve) through a putative karyoskeleton (red). In (**B**) the chromatin is shown directly interacting with the nuclear lamina and a splicing factor compartment (green).

and specific contacts with the karyoskeleton. Testing this hypothesis will require a better understanding of the composition of the karyoskeleton and the mechanistic basis of the interactions. In the second model, the targeting is dependent upon the regional stability of the chromosome. Regions of heterochromatinization are least likely to decondense under these conditions because of stronger internucleosomal and fiber–fiber interactions. In contrast, regions of euchromatin, such as those rich in acetylation, are more likely to unfold in response to an applied force. Experiments that simultaneously quantify changes in gene expression in response to changing cell shape, map the positions of these responsive regions of the genome, and measure the biochemical properties of these regions relative to their surroundings will be necessary to advance our mechanistic understanding of mechanotransduction to the genome.

CONCLUSIONS

At every other scale, we accept that both the chemical and the physical make important contributions to the process of life. At the cellular scale, however, chemistry has been considered to be almost exclusively responsible for the phenomena that we observe in cells and which define cell phenotype. This bias may, in part, be based upon the technologies available to study cellular phenomena. With the advent of micropatterned surfaces and the development of 3D tissue culture models, we may be at a turning point. The identification and characterization of the LINC and NELSC complexes has highlighted physical phenomena that reflect communication between the nucleus, the cell, and, by extension, the ECM. These complexes provide a mechanistic explanation for changes in nuclear shape and chromatin structure that

are observed in many human cancers. They may also aid in understanding how changes in the cellular microenvironment through tissue remodeling in tumors contribute to changes in cell phenotype. The integrins and other extracellular matrix (ECM) receptors are the cellular interface with the microenvironment and a platform from which physical and biochemical signaling is initiated. The cellular microenvironment is dynamic; cells affect themselves and their neighbors physically by altering tissue ECM tension. They physically transmit the information about their own internal tension in three dimensions. Each cell can alter the ECM chemically through secretory additions, enzyme degradation and physical distortion thereby providing a mechanism where cells can both affect and be effected by changes in the microenvironment. There is no doubt that biochemical signals, which are also transmitted in the environment and within the cell both actively and by diffusion, play an important role in defining cellular phenotype and the underlying gene expression profiles. Now that some of the key cellular machinery that mechanically integrates the nucleus and the genome to the entire cell and the microenvironment have been identified, we can begin to experimentally test and define the functional contribution of cellular mechanics to the definition of cell phenotype. Since many of the hallmarks of human cancers are recognized based on morphological changes that have roots in biophysical changes, the evolution of this field has the potential to contribute a great deal to bridging molecular, cellular, and tissue biology and lead to new approaches in the treatment of human cancers.

ACKNOWLEDGMENTS

We would like to thank Dr. Brian Burke and J.B. Rattner for communicating unpublished results. The experimental work was supported by operating grants from the Canadian Institutes of Health Research and the National Cancer Institute of Canada. MJH is a senior scholar of the Alberta Heritage Foundation for Medical Research.

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