Gene Expression in the Third Dimension: The ECM-nucleus Connection

Virginia A. Spencer, Ren Xu & Mina J. Bissell

Authors' Affiliation:

Life Sciences Division, Lawrence Berkeley National Laboratory,
1 Cyclotron Road, MS 977R225A,
Berkeley, CA 94720, USA

Corresponding Authors:

V.A. Spencer
Email: vaspencer@lbl.gov

M.J. Bissell
Email: mjbissell@lbl.gov

Abstract

Decades ago, we and others proposed that the dynamic interplay between a cell and its surrounding environment dictates cell phenotype and tissue structure. Whereas much has been discovered about the effects of extracellular matrix molecules on cell growth and tissue specific gene expression, the nuclear mechanisms through which these molecules promote these physiological events remain unknown. Using mammary epithelial cells as a model, the purpose of this review is to discuss how the extracellular matrix influences nuclear structure and function in a three-dimensional context to promote epithelial morphogenesis and function in the mammary gland.

Keywords

Extracellular matrix
Gene expression
Nuclear structure

Abbreviations

ECM	extracellular matrix
LrECM	laminin-rich extracellular matrix
CTs	chromosome territories
ES	embyronic stem
MEC	mammary epithelial cell
Col-IV	collagen type IV
Col-I	collagen type I
LN1	laminin type I
LN5	laminin type V
BM	basement membrane
ILK	integrin-linked kinase
PrlR	prolactin receptor
STAT5	signal transducer and activator of transcription 5
KASH	Klarsicht, ANC-1, Syne Homology
SUN	Sad1p, UNC-84
Jak2	Just-Another-Kinase 2
BCE-1	bovine β-casein ECM response element
NuMA	nuclear mitotic apparatus
PML	promyelocytic leukemia

Introduction

The average diameter of a nucleus is 10–15 microns, yet compressed within this organelle is about 2 m of DNA that is intricately interwoven with a plethora of RNA and protein [1–3]. Such complexity and molecular crowding leaves one to wonder exactly how the nucleus functions with such efficiency and precision throughout the life and differentiation of a cell.

Over the years, researchers have come to realize that the nucleus has an incredible degree of architectural organization which plays a fundamental role in its functional output. Chromatin is organized into discrete domains referred to as chromosome territories (CTs) that generally contain genepoor sequences along their exterior regions and gene-rich regions in the interior which are known to be AT-rich [4–7]. The organization of CTs and the expression of DNA are influenced by an underlying protein-and RNA-rich scaffold referred to as the nuclear matrix, scaffold or substructure [8]. This substructure serves as a platform for a host of nuclear metabolic processes and chromatin modifying enzymes [9], many of which are localized into discrete domains throughout the nucleoplasm [10–12].

Despite the fact that the nucleus is highly structured, it is still a dynamic organelle. The most extreme case of this plasticity can be found in pluripotent embyronic stem (ES) cells. When compared to their lineage-committed daughter cells, the chromatin in ES cells is homogenously decondensed, transcriptionally active on a global scale, loosely associated with histones and architectural chromatin binding proteins such as heterochromatin protein 1, deficient in DNA methylation, and enriched in epigenetic histone modifications indicative of actively transcribed genes [13– 15]. During ES cell differentiation, the ratio of euchromatin to heterochromatin declines, the expression of chromatin remodeling proteins and general transcription factors drops, and transcriptional activity decreases [13]. Despite these dramatic changes, the nucleus of a differentiated cell retains some transcriptional activity through the action of specific chromatin and nuclear factors which dynamically associate with discrete regions of the genome [16], ultimately tailoring a transcriptional profile that favors tissue-specific function over growth. But what are the exact factors that guide the nucleus into this most advanced stage of differentiation, also referred to as functional differentiation?

Almost three decades ago, we proposed that the dynamic reciprocity between a cell and its surrounding environment dictates tissue structure and function [17]. Since then, we and others have shown that the extracellular matrix (ECM) plays an important role in regulation of nuclear events important for growth arrest and mammary-specific gene expression. In this review, we briefly discuss how the ECM influences nuclear architecture and how these changes may impact the transcriptional profile of mammary epithelial cells (MEC) to promote functional differentiation.

The Mammary Gland: A Versatile Model for Studying the ECM-nucleus Connection

Unlike all other organs in the body, the mammary gland and, more specifically the mammary epithelium, develops post-natally and undergoes periods of active remodeling during estrus and pregnancy [18–21]. During puberty, MECs proliferate as an arborous, ductal network through

regions of actively remodeled ECM in the mammary fat pad [22, 23]. Once this ductal network reaches the end of the fat pad, the epithelial cells stop proliferating and become surrounded by a specialized collagen type IV (Col-IV)-, laminin type V (LN5)- and type I (LN1)-rich ECM (referred to as a basement membrane: BM) [22–25]. This state of quiescence becomes interrupted during pregnancy when the mammary gland ECM undergoes additional rounds of remodeling and the epithelial cells proliferate into functionally differentiated, growth-arrested acinar structures that produce milk proteins in preparation for lactation [26]. It is because of these developmental changes that the mammary gland is an ideal model for studying how BM molecules interact with the nucleus to influence its form and function.

The ECM is an Inducer of Tissue-specific Gene Expression

Using the floating collagen type I (Col-I) gel methodology developed by Mikopolous and Pito [27], it was demonstrated that the physical properties of the extracellular substratum can induce dramatic changes in cell shape and tissue specific gene expression. More specifically, MECs cultured on a Col-I gel which was adhered to a plastic surface assumed a flattened and spread morphology. When the contact between this gel and its surface was disrupted, MECs became cuboidal in shape and began to express milk proteins [28–31]. In a follow-up study by Streuli and Bissell, MECs cultured in floating, but not attached, Col-I gels were shown to produce and deposit a Col-IV- and LN1-rich ECM within a time frame that correlated with the onset of milk protein production [32]. Later studies revealed that LN1 was the main BM component mediating milk protein expression [33] but its mechanism of action proved to be quite complex, involving both biochemical and mechano-transduction events [34].

Despite this complexity, Muschler and colleagues identified distinct cell surface receptors through which LN1 initiates biochemical, and morphological events that are important for tissue-specific gene expression [35, 36]. One of these receptors is dystroglycan, a LN1 co-receptor that facilitates laminin anchoring and assembly [37], thereby allowing for efficient β-casein production [36]. β1- and α6β4-integrins are also key receptors that bind to LN1 and mediate biochemical signaling pathways involved in β-casein expression [35].

Later studies addressing ECM signaling and milk protein expression showed that LN1 binding to the β1-integrin receptor signals through integrin-linked kinase (ILK) to promote the activation of the Rho GTPase, RAC1 [38]. Activated Rac1 then works in combination with prolactin to mediate activation of the prolactin receptor (PrlR)/Signal Transducer and Activator of Transcription 5 (STAT5) signaling cascade which subsequently phosphorylates and promotes the nuclear translocation of STAT5, a key transcription factor for β-casein gene transcription [39–41].

The Cytoskeleton and the ECM-nucleus Connection

When a cell interacts with its surrounding environment, it transmits both mechanical and biochemical signals from its surface to its nucleus interior. This communication relies heavily on the cytoskeleton, a structural network composed of actin microfilaments, microtubules and intermediate filaments. The cytoskeleton bridges and relays signals from the extracellular matrix to the nucleus through its interaction with focal adhesion complexes at extracellular matrix

receptors [42, 43], and Klarsicht, ANC-1, Syne Homology (KASH)-domain proteins at the outer nuclear envelope [44–46]. KASH domain proteins are targeted to the outer nuclear envelope through their interactions with inner nuclear membrane components referred to as Sad1p, UNC-84 (SUN)-domain proteins, which, in turn, interact with chromosomes and nuclear components such as lamins [44, 45, 47]. Thus, the cell responds to the ECM as a physically integrated unit.

Evidence for the physical connection between a cell's ECM receptor and the nucleus was elegantly demonstrated by Ingber and colleagues when the mechanical tug of an integrin receptor lining the surface of an epithelial cell induced alterations in cytoskeletal organization, distortions in nuclear shape and a redistribution of nucleoli along the axis of applied tension [48]. In addition, Lammerding and colleagues have shown that disruptions in this mechanical continuity through mutation of the lamin A/C gene result in embryonic fibroblasts that display a decrease in stiffness and an increase in nuclear deformation in response to mechanical strain [49].

The ECM can induce vast changes in cellular and cytoskeletal organization. For example, when MECs are cultured in a three-dimensional LN1-rich ECM (lrECM), they organize into polar, acinar-like structures or spheroids [50] and their cytoskeleton rearranges into a cortical network [34] that is required for PrlR downstream signaling to STAT5 [51]. Recently we have shown that exposure of MECs to a lrECM promotes the recruitment of the prolactin receptor to the basal surface of spheroids where it is accessible for binding to prolactin in the surrounding milieu (Fig. 1) [41]. Once these structural changes have occurred, prolactin can bind to its receptor and promote Just-Another-Kinase 2 (Jak2)-mediated phosphorylation of Stat5. It is important to note that prolactin can induce STAT5 phosphorylation independently of LN1 treatment [41]. This event, however, is short-lived and must be sustained for milk protein expression [41].

In order for LN1 to promote these structural events, the extracellular environment must provide a matrix which is compliant. We have shown recently that the laminin-rich environment of the mammary gland is relatively 'soft' and that this property is physically propagated in MECs through actin depolymerization events which are mediated by integrin receptors [52]. Increasing the stiffness of Col-I gels (by glutaraldehyde fixation) [31] or LN1 gels (through Col-I supplementation) [52] attenuated β-casein expression, suggesting that matrix flexibility is required for cytoskeletonmediated cellular and intercellular rearrangements which are essential for tissue-specific gene expression.

Tissue-specific Gene Expression and the ECM-nucleus Connection

Perhaps one of the most convincing early pieces of evidence linking the ECM to gene expression was the identification of an ECM-response element in the promoter region of several ECMresponsive genes from different species [53]. Interestingly, an experiment studying activation of the bovine β-casein ECM response element, BCE-1, in transiently and stably-transfected MECs showed that this element must be stably integrated into the genome of a MEC in order to be activated by LN1 and prolactin [39]. The fact that BCE-1 requires integration into the genome to become transcriptionally active suggests that chromatin structure, and the proximity of this sequence to its target transcription factors, chromatin remodeling factors and

the basal transcriptional machinery likely play an essential role in the ability of LN1 to activate this element. In support of this, histone deacetylase inhibitor treatment of cells stably transfected with BCE-1 was sufficient to activate the BCE-1element in the absence of LN1 [39].

Quiescence and the ECM-nucleus Connection

A MEC must stop dividing for it to undergo functional differentiation (i.e. produce milk proteins). Prior studies from our laboratory have shown that, in addition to promoting milk protein expression, LN1 causes MECs to growth arrest [54, 55]. Thus, the role of LN1 in nuclear events linked to functional differentiation extends far beyond its role in milk protein expression.

The mechanism for growth inhibition is unknown, but the phenotypic effects of LN1 on nuclear organization provide clues which may eventually reveal the molecular events responsible for this physiological event. For example, studies performed on MECs have shown that LN1 dramatically increases epigenetic events linked to gene silencing such as histone deacetylation, chromatin condensation, and DNA methylation [56–58]. In addition, an analysis of the mRNA expression profile of MECs showed that LN1 down-modulates twice the number of genes that it up-regulates [56]. Thus, the ECM induces profound changes in chromatin structure and nuclear factor organization that are suggestive of a large-scale suppression of gene activation. Whether the repertoire of affected genes includes those important for cell growth, remains to be shown.

The ability of LN1 to promote the formation of a transcriptionally repressive nuclear environment may, in part, stem from its effect on nuclear factor distribution. LN1 treatment causes proteins in the nuclei of MECs such as the splicing factor SC-35, Tin2 and nuclear mitotic apparatus (NuMA) to coalesce into large foci [59–61]. The purpose of this coalescence in mammary cells remains unknown. In a previous study, exogenous nuclear factors which were over-expressed formed large foci that co-localized with promyelocytic leukemia (PML) bodies [62]. PML bodies have been proposed to be nuclear factor storage sites [63]. The disruption of LN1-induced NuMA foci leads to alterations in chromatin structure, re-initiation of cell growth and production of LN1-degrading metalloproteinases [61]. Thus, it is possible that the effect of LN1 on SC35, NuMA and Tin2 represents a regulatory mechanism through which this ECM molecule can control the availability of these factors and their ability to sustain or promote nuclear functions important for cell growth. Alternatively, LN1-mediated chromatin condensation could passively promote the coalescence of nuclear factors situated next to genes important for cell growth. In support of this, nuclease treatment of chromatin has been shown to disturb the integrity and stability of PML bodies [64].

LN1 could also induce growth arrest by causing specific subsets of proteins to become post-translationally modified in ways that alter their nuclear localization, activity, turnover, and interactions with other proteins. One modification that stands out in particular is sumolation because it is required for the assembly of PML bodies [65], as well as the recruitment of DNA-modifying and transcription factors to these nuclear compartments [66, 67]. In the majority of cases, sumolation negatively regulates the activity of transcription factors [68]. Thus, inducing protein sumolation may be one way in which LN1 inhibits the activity and promotes the coalescence of specific nuclear factors required for cell growth.

From Laminin to Lamin: Connecting the Dots

The findings discussed above establish a strong connection between the ECM and the nucleus. They also demonstrate that MEC acinar morphogenesis and milk protein expression are multi-stepped, complex processes which require several levels of control [69–71] (Fig. 2). However, much remains to be learned about how the extracellular environment moulds the nuclear landscape in a three-dimensional context to promote functional differentiation. In addition, how the ECM impacts nuclear structure and function to influence cancer development remains a mystery. Indeed, a role for the ECM in tumorigenesis is likely since loss of tissue structure is considered one of the hallmarks of cancer development [72]. Through further advancements in our knowledge of the ECM-nucleus connection, we stand to gain valuable insight into the cellular processes that govern normal and tumorigenic cell behavior: insight that will likely reveal new and effective avenues for disease intervention.

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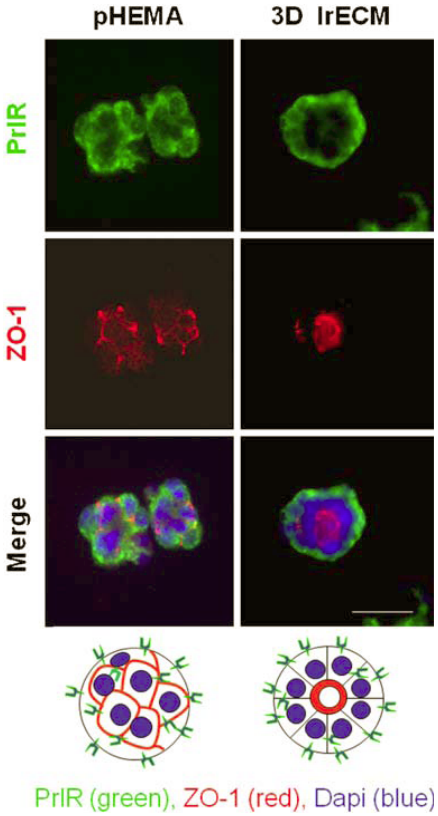
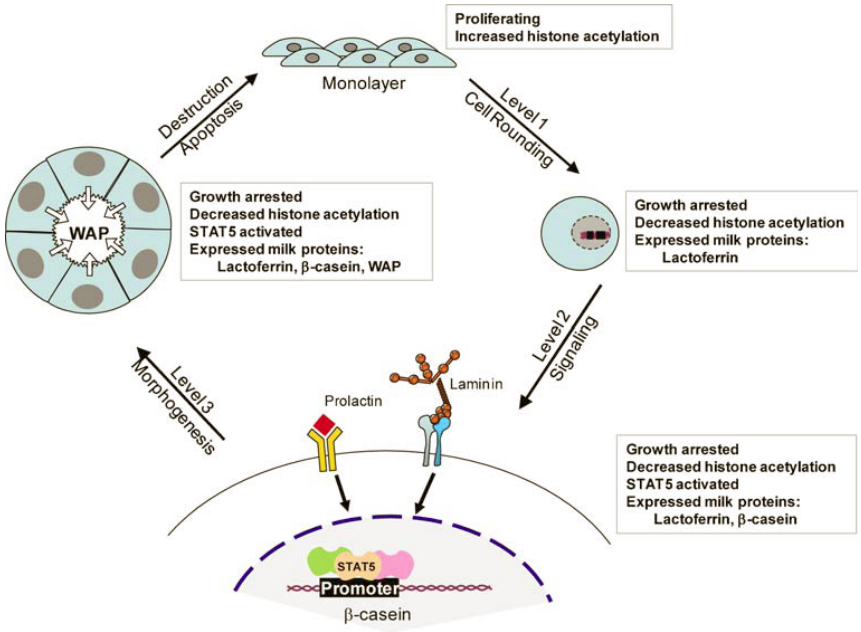
Figures

FIGURE 1



LN1 alters spheroid polarity to induce changes in prolactin receptor (PrlR) cell surface localization that promote its accessibility to basally distributed ligand. Immunofluorescence analysis of the PrlR and ZO-1, a marker of apical polarity, in EpH4 MEC spheroids that were pre-clustered on a non-adhesive substratum and treated with medium containing prolactin (right) or prolactin and 2% lrECM for 24 h. Scale bar represents 25 μm. Adapted with permission from [41].

FIGURE 2



Schematic illustration of the different levels through which LN1 exerts control over mammary gland function and structure. When cultured on a two-dimensional surface, mammary epithelial cells proliferate, and express high levels of histone acetylation. The transition of a MEC from a two-dimensional surface to a three dimensional lrECM involves changes in cell shape which alone can activate the expression of specific genes such as the milk protein lactoferrin [34, 73]. As these changes occur, the cells begin to arrest growth and to decrease their steady state histone acetylation levels [56, 58]. Exposure to prolactin and laminin type I or LN1 induces biochemical signaling events that sustain the activation of STAT5 [41], a transcription factor which is required for β-casein expression [40, 74]. Once activated, STAT5 translocates to the nucleus, binds to the β-casein promoter and associates with additional transcription factors and chromatin remodeling complexes that are required for RNA Pol II recruitment [75]. As the duration of exposure to an intact laminin-rich BM increases, MECs continue to alter their gene expression profile and organize into acinar structures that express whey acidic protein (WAP). These structures are eventually destroyed by BM-degrading metalloproteases during the involution process. Adapted with permission from [53, 69, 71].