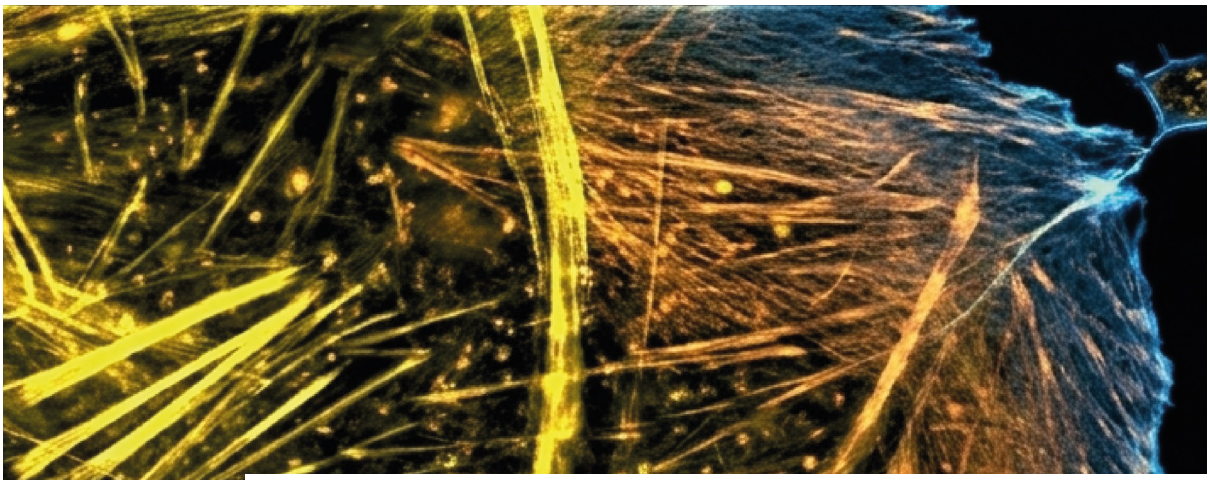
Decoding the Matrix: Multiscale Mechanosensing and Mechanical Memory in Fibroblasts

Packirisamy, Swathi

2026

Document Version:
Publisher's PDF, also known as Version of record



Decoding the Matrix:

Multiscale Mechanosensing and Mechanical Memory in Fibroblasts

SWATHI PACKIRISAMY
DEPARTMENT OF CLINICAL SCIENCES | FACULTY OF MEDICINE | LUND UNIVERSITY

Decoding the Matrix:
Multiscale Mechanosensing and Mechanical Memory in Fibroblasts

Decoding the Matrix: Multiscale Mechanosensing and Mechanical Memory in Fibroblasts

Swathi Packirisamy

LUND
UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy at the Faculty of medicine at Lund University to be publicly defended on 10th of September at 9.00 in Belfragesalen, Biomedical Centre (BMC), Lund, Sweden

Faculty opponent:
Dr. Yekaterina Miroshnikova
Max Planck Institute, Germany

Organization: LUND UNIVERSITY

Document name: Doctoral Dissertation **Date of issue:** 2026-09-10

Author(s): Swathi Packirisamy **Sponsoring organization:**

Title and subtitle:

Decoding the Matrix: Multiscale Mechanosensing and Mechanical Memory in Fibroblasts

Abstract:

Cancer progression is profoundly influenced by the dynamic interactions between cancer cells and their surrounding tumour microenvironment (TME). A defining feature of the TME is the progressive remodelling of the extracellular matrix (ECM), which becomes stiffer and structurally altered during disease progression. These changes regulate cell behaviour and contribute to tumour growth, invasion and metastasis. Fibroblasts are central mediators of this process, sensing ECM mechanics through integrin-based focal adhesions (FAs) and responding by altering their activation state and remodelling the surrounding matrix. Increasing evidence suggests that fibroblasts can acquire mechanical memory and remain active even after the initiating mechanical stimulus has been removed, driving pathological ECM remodelling and disease progression. However, the exact molecular mechanisms by which fibroblasts sense the ECM and become persistently activated remain poorly understood.

The aim of this thesis was to investigate how fibroblasts sense the mechanical properties of the ECM and how mechanosensing is coupled to persistent fibroblast activation. Using cell biology, imaging, computational modelling and molecular approaches, this work demonstrates that the nanoscale organization of FA components is a key regulator of mechanosensing and identifies septin-7 as a spatial regulator of FA maturation. Furthermore, it shows that integrin-mediated ECM sensing is linked to chromatin remodelling, providing a mechanistic basis for the establishment of persistent fibroblast activation through mechanical memory.

Key words: Extracellular matrix, fibroblasts, cancer associated fibroblasts, mechanosensing, integrins, focal adhesions, mechanical memory

Language: English **Number of pages**: 91 (excluding papers)

ISSN and key title: 1652-8220

ISBN: 978-91-8021-901-3

Recipient's notes Price Security classification

Signature Date: 2026-07-30

Decoding the Matrix: Multiscale Mechanosensing and Mechanical Memory in Fibroblasts

Swathi Packirisamy

LUND
UNIVERSITY

Cover image: Microscopy image taken by the author and colorized using Google Gemini

Published by:

Department of Clinical sciences

Faculty of Medicine

Lund University

ISSN 1652-8220

ISBN 978-91-8021-901-3

Lund University, Faculty of Medicine Doctoral Dissertation Series 2026:103

Printed in Sweden by Media-Tryck, Lund University, Lund, 2026

Media-Tryck is a Nordic Swan Ecolabel certified provider of printed material. Read more about our environmental work at www.mediatryck.lu.se

MADE IN SWEDEN

The most exciting phrase to hear in science is
not "Eureka!" but "That's funny..."
- Isaac Asimov,

Table of Contents

List of figures

Author declaration: I, the author, take full responsibility for the content presented in this thesis. This thesis has been prepared with assistance from generative AI tools for language assistance and writing suggestions. All the text has been reviewed by the author.

Abbreviations

ECM	Extracellular matrix
TME	Tumour microenvironment
FAs	Focal adhesions
CAFs	Cancer associated fibroblasts
TGF-β	Transforming growth factor-beta
αSMA	alpha-smooth muscle actin
FAK	Focal adhesion kinase
YAP	Yes-associated protein
LINC	Linker of nucleoskeleton and cytoskeleton
LADs	Lamin associated domains
HATs	Histone acetyltransferases
HDACs	Histone deacetylases
FHOD1	Formin homology domain containing protein 1
mDia2	mammalian Diaphanous-related formin-2

Abstract

Cancer progression is profoundly influenced by the dynamic interactions between cancer cells and their surrounding tumour microenvironment (TME). A defining feature of the TME is the progressive remodelling of the extracellular matrix (ECM), which becomes stiffer and structurally altered during disease progression. These changes regulate cell behaviour and contribute to tumour growth, invasion and metastasis. Fibroblasts are central mediators of this process, sensing ECM mechanics through integrin-based focal adhesions (FAs) and responding by altering their activation state and remodelling the surrounding matrix. Increasing evidence suggests that fibroblasts can acquire mechanical memory and remain active even after the initiating mechanical stimulus has been removed, driving pathological ECM remodelling and disease progression. However, the exact molecular mechanisms by which fibroblasts sense the ECM and become persistently activated remain poorly understood.

The aim of this thesis was to investigate how fibroblasts sense the mechanical properties of the ECM and how mechanosensing is coupled to persistent fibroblast activation. Using cell biology, imaging, computational modelling and molecular approaches, this work demonstrates that the nanoscale organization of FA components is a key regulator of mechanosensing and identifies septin-7 as a spatial regulator of FA maturation. Furthermore, it shows that integrin-mediated ECM sensing is linked to chromatin remodelling, providing a mechanistic basis for the establishment of persistent fibroblast activation through mechanical memory.

Popular summary

Every cell in our body constantly receives thousands of signals that help it understand what is around it. To function properly, cells must communicate not only with each other but also with the non-cellular network that surrounds them, known as the extracellular matrix (ECM). These signals can be biochemical in nature, carried by small messenger molecules, or mechanical, arising from the physical forces cells experience when they interact with neighbouring cells or the ECM. This thesis focuses on how cells sense and respond to these mechanical signals.

The ECM is a dynamic scaffold whose physical properties vary between tissues. For example, brain cells reside in soft environments while bone cells are surrounded by a much stiffer matrix. In addition to stiffness, the ECM also differs in its composition and architecture. Cells explore these properties using specialised structures called focal adhesions that act like tiny molecular hands that attach to the ECM. By binding to ECM components, cells can identify the composition of the ECM and the local stiffness by pulling and pushing on them. This helps them to build a picture of their mechanical surroundings.

The information gathered at FAs is transmitted through the cell's internal scaffolding, the cytoskeleton, to the nucleus, where the cells genetic material is housed. At the same time, mechanical forces trigger biochemical signalling through a process known as mechanotransduction. Together, these signals allow the nucleus to regulate genes that control important cellular behaviours such as movement, growth and survival. When cells are exposed to the same mechanical environment for long periods, they can develop a form of 'mechanical memory'. Even if their surroundings later change, they may continue behaving as though they are still in the original environment. This can have harmful consequences as seen in cancer where fibroblasts become activated by stiff environment of the tumour. Remarkably, they can remain activated after leaving the tumour, continuing to remodel surrounding tissue and promote tumour growth and metastasis.

This thesis investigates how fibroblasts detect mechanical cues from their environment and how these signals are converted into long lasting changes in cell behaviour. We show that the nanoscale organization of proteins within focal adhesions plays a central role in regulating how cells sense mechanical forces and that septins, a group of cytoskeletal proteins, help spatially organize focal adhesions. Finally, we also found that the FA receptor, integrin, mediated mechanical sensing is linked to chromatin remodelling to establish mechanical memory. Together, these findings provide new insights into how cells translate physical forces into biological responses and help advance our understanding of diseases involving abnormal tissue remodelling, including cancer.

Populärvetenskaplig sammanfattning

Varje cell i vår kropp tar ständigt emot tusentals signaler som hjälper den att förstå sin omgivning. För att fungera korrekt måste celler kommunicera inte bara med varandra utan också med det nätverk som omger dem – den så kallade extracellulära matrisen (ECM). Dessa signaler kan vara av biokemisk natur, förmedlade av små budbärarmolekyler, eller mekaniska, härrörande från de fysiska krafter som uppstår när celler interagerar med grannceller eller ECM. Denna avhandling fokuserar på hur celler känner av och svarar på dessa mekaniska signaler.

ECM är en dynamisk stödstruktur vars fysiska egenskaper varierar mellan olika vävnader. Exempelvis befinner sig hjärnceller i en mjuk miljö, medan benceller omges av en betydligt styvare matris. Utöver styvhet skiljer sig ECM även åt vad gäller sammansättning och struktur. Celler utforskar dessa egenskaper med hjälp av specialiserade strukturer som kallas fokala adhesioner; dessa fungerar som små molekylära händer som fäster vid ECM. Genom att binda till komponenter i ECM kan cellerna identifiera matrisens sammansättning och lokala styvhet genom att dra i och trycka mot dem. Detta hjälper dem att skapa sig en bild av sin mekaniska omgivning.

Informationen som samlas in vid de fokala adhesionerna överförs via cellens inre stödstruktur – cellskelettet – till cellkärnan, där cellens genetiska material finns. Samtidigt utlöser mekaniska krafter biokemisk signalering genom en process som kallas mekanotransduktion. Tillsammans gör dessa signaler det möjligt för cellkärnan att reglera gener som styr viktiga cellulära processer såsom rörelse, tillväxt och överlevnad. När celler utsätts för samma mekaniska miljö under lång tid kan de utveckla en form av ”mekaniskt minne”. Även om omgivningen senare förändras kan de fortsätta att bete sig som om de fortfarande befann sig i den ursprungliga miljön. Detta kan få skadliga konsekvenser, vilket syns vid cancer där fibroblaster aktiveras av tumörens styva miljö. Anmärkningsvärt nog kan de förbli aktiverade även efter att de lämnat tumören, och fortsätta att omforma omgivande vävnad samt främja tumörtillväxt och metastasering.

Denna avhandling undersöker hur fibroblaster känner av mekaniska signaler från sin omgivning och hur dessa signaler omvandlas till långvariga förändringar i cellens beteende. Vi visar att organiseringen av proteiner på nanonivå inom fokala adhesioner spelar en central roll för hur celler känner av mekaniska krafter, och att septiner – en grupp proteiner i cellskelettet – bidrar till att organisera de fokala adhesionerna rumsligt. Slutligen fann vi också att den mekaniska avkänning som förmedlas av receptorn integrin (vid fokala adhesioner) är kopplad till omformning av kromatinet för att etablera ett mekaniskt minne. Tillsammans ger dessa resultat nya insikter i hur celler omvandlar fysiska krafter till biologiska svar, och de bidrar

till att öka vår förståelse för sjukdomar som involverar onormal vävnadsombyggnad, däribland cancer.

Publications

I. Grudtsyna V, **Packirisamy S**, Bidone TC, Swaminathan V. Extracellular matrix sensing via modulation of orientational order of integrins and F-actin in focal adhesions. Life Sci Alliance. 2023 Jul 18;6(10):e202301898. doi: 10.26508/lsa.202301898

II. Sturgess W, **Packirisamy S**, Geneidy R, Nordenfelt P, Swaminathan V. ECM-dependent regulation of septin 7 in focal adhesions promotes mechanosensing and functional response in fibroblasts. iScience. 2024 Nov 9;27(12):111355. doi: 10.1016/j.isci.2024.111355

III. **Packirisamy S**, André O, Fan Z, Nordenfelt P, Swaminathan VS. ECM-Stiffness Mediated Persistent Fibroblast Activation Requires Integrin and Formin Dependent Chromatin Remodeling. Adv Sci (Weinh). 2026 Jun;13(34):e17631. doi: 10.1002/advs.202517631.

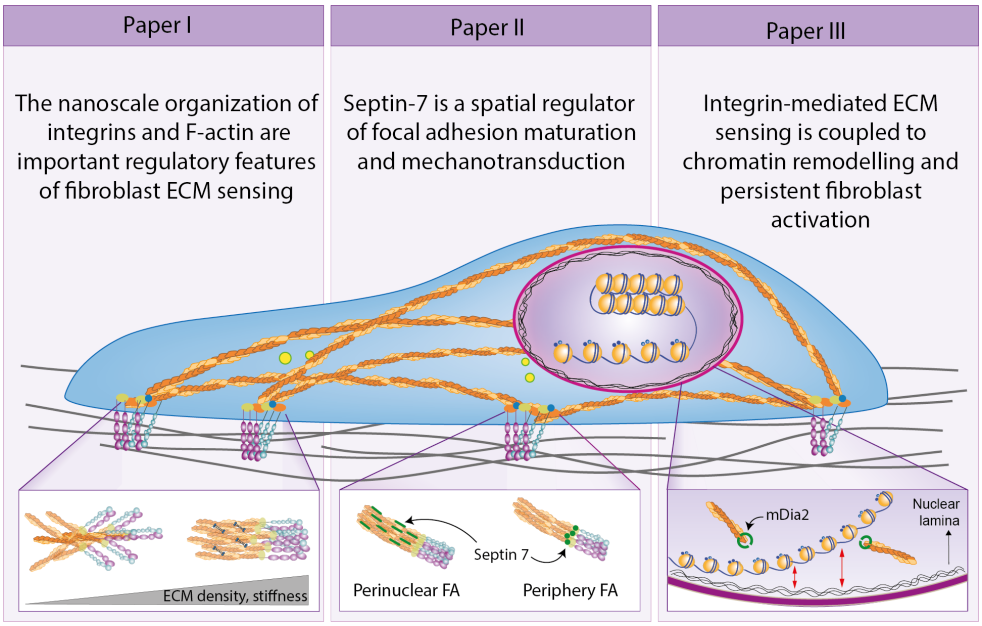
Authors contributions to publications

I. Major part in designing, planning, performing experimental work and writing.

II. Contributed to planning, performing experimental work and manuscript editing.

III. Major part in designing, planning and writing. Performed all the experimental work.

Thesis at a glance



Introduction

Cancer development and progression are increasingly recognized as processes that are shaped not only by malignant cells, but also by their surrounding tumour microenvironment (TME). The various cellular and non-cellular components of the TME influence tumour growth, invasion and metastasis through complex biochemical and biophysical interactions. Among these, the extracellular matrix (ECM) has emerged as an important regulator of cell behaviour where extensive alterations to the ECM result in stiffer microenvironments, a hallmark of solid tumours and disease progression. These changes involving ECM stiffness, architecture and composition are intricate, multidimensional and act over long timescales.

Fibroblasts are central mediators of ECM remodelling. They sense changes in ECM mechanics through specialized adhesion complexes called focal adhesions (FAs). FAs help translate physical cues into intercellular biochemical signals that alter their gene expression profiles and ultimately their activation states. Activated fibroblasts can then contribute to the progressive stiffening of the TME and create a positive feedback loop between ECM stiffness and fibroblast activation. Recent evidence further suggests that mechanical signals from stiff ECMs can induce persistent fibroblast activation where fibroblasts retain a memory of their past environmental experience and remain active even when the initial activating stimulus is removed. The acquisition of this kind of mechanical memory may be the driving force behind sustained pathological ECM remodelling and disease progression.

While there has been significant progress in understanding how cell types including fibroblasts sense and respond to the mechanical ECM, several questions remain unanswered, specifically in the context of the TME. These include how complex ECM cues, such as stiffness, density and architecture, are sensed and encoded by fibroblasts, and how this sensing leads to persistent activation states. Addressing these questions will provide fundamental insights into the mechanistic workings of ECM adhesion receptors and the acquisition of environmental memory. Understanding these processes will have implications that extend well beyond cancer as aberrant ECM remodelling and mechanosignalling are central features of a number of diseases such as fibrosis, connective tissue disorders and degenerative joint and vascular diseases.

This thesis explores the mechanisms by which fibroblasts sense and respond to mechanical properties of the ECM and the molecular mechanisms that link mechanosensing to persistent fibroblast activation. The work demonstrates that the nanoscale organization of focal adhesion components constitutes an important regulatory feature of ECM sensing and identifies septin-7 as a previously unrecognised spatial regulator of focal adhesion maturation. Finally, it reveals how integrin-mediated ECM sensing is coupled to chromatin remodelling to establish persistent fibroblast activation through mechanical memory. Together, these findings provide new mechanistic insights into how fibroblasts decode distinct ECM cues and translate them into stable cellular responses.

To outline the key concepts, this chapter begins with a broad overview of the ECM and TME and the key cell types responsible for their formation and maintenance, i.e. fibroblasts and CAFs. It then introduces FAs and the major mechanotransduction pathways that enable cells to sense and respond to ECM mechanics. Finally, the chapter discusses how signals originating at FAs are transmitted to the nucleus to regulate gene expression and the probable molecular mechanisms underlying the establishment of mechanical memory.

The Extracellular matrix and fibroblasts in health and disease

Biological systems are governed by both biochemical and mechanical cues. While the role of biochemical cues such as growth factors and cytokines has been extensively studied over the past few years, how mechanical cues such as stiffness, tension, compression, shear stress and strain regulate cell behaviour is a relatively new and emerging area of research[1-3]. Understanding how cells sense and respond to these signals is critical to understanding both physiological processes such as tissue development and wound healing and pathological processes like cancer[4, 5]. In this context, the extracellular matrix and fibroblasts play important roles.

The extracellular matrix

The ECM is the non-cellular component that fills up the intercellular spaces of all multicellular organisms. It is composed primarily of structural proteins, polysaccharides and water, though its composition varies between tissues, enabling it to meet distinct biological demands. Consequently, it functions not only as a structural scaffold providing mechanical support to tissues, but also as an active component of the cell microenvironment, directing cell behaviours, tissue homeostasis and organ development[6, 7].

Properties and functions of the ECM

Each ECM component contributes distinct structural and signalling functions to the matrix, but it is their combined activity that determines cell behaviours[6-8] (Figure 1). Structural proteins such as fibrillar collagen I, II, III and V form the primary load bearing network and are responsible for maintaining the structural integrity of the ECM. Their mechanical properties such as fibre diameter and elasticity are further regulated through post-translational crosslinking[9]. In contrast, non-fibrillar collagens such as collagen IV, VII and XV are found in the basement membrane, a specialized form of the ECM in epithelial and endothelial tissues that acts as a structural barrier between them and the underlying stroma[10]. Fibronectin is another structural protein that forms large assemblies. It responds to cell generated forces and can transition from compact folded states to stretched conformations that increase protein rigidity but also reveal cryptic binding sites that regulate cellular adhesion and growth factor accessibility[11]. Resistance to compressive forces are provided by proteoglycans that have high water retention properties and serve as trap for growth factors and other biochemical cues[12]. Finally, glycoproteins such as Tenascin-C further modulate ECM function during wound repair by weakening cell adhesion and promoting cell migration[13].

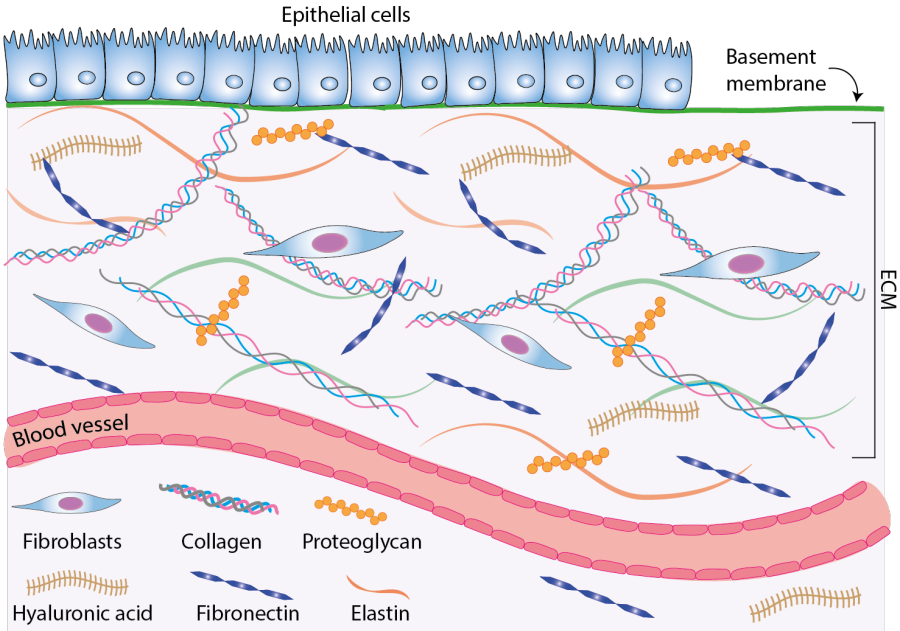


Figure 1: The extracellular matrix
The ECM is a dynamic network of macromolecules that surrounds and supports cells in tissues. It is made up of fibrous proteins and the ground substance. Fibrous proteins such as fibronectin, collagen and elastin provide tensile strength, elasticity and cellular adhesion sites. The ground substance is a hydrated gel like material that fills the space between cells and fibres and is composed of proteoglycans and glycosaminoglycans like hyaluronic acid. Image created with Adobe Illustrator.

Depending on the composition and organization of these components, the ECM can have distinct biochemical and mechanical properties that govern how cells interact with their microenvironment. The ease with which the matrix can be remodelled and reorganised to enable cell migration depends on its overall stiffness[14]. Stiffness can also regulate cell differentiation through the activity of growth factors. For example, transforming growth factor-beta (TGF-β) is stored in the ECM in its latent form and activated through cellular forces that can only be generated on stiff ECMs[15, 16]. Another physical property of the ECM that regulates cell migration is its topology, such as fibre alignment and density that provide physical guidance cues to direct cell migration. This has been observed during development and wound healing where aligned collagen fibres can promote anisotropic cell movement but can also facilitate invasive migration in cancer[17-20]. Furthermore, the availability of ligands and anchorage sites in the matrix can also regulate cell division[21]. During development and wound healing, these physical properties of the ECM are dynamically altered to regulate cell behaviours and functions.

ECM dynamics and remodelling in health and disease

ECM homeostasis is maintained through a tightly regulated remodelling process and is a fine balance between matrix deposition, crosslinking, degradation and cell-mediated mechanical reorganization. Matrix stabilization is driven by ECM crosslinking enzymes such as peroxidasin, lysyl oxidases (LOX) and transglutaminases whereas ECM degradation is mediated via proteolytic cleavage by MMPs, serine proteases and cathepsins[22-24]. In addition to altering the ECM structure, protein degradation also releases bioactive fragments that modulate cellular behaviour and ECM remodelling. This is seen during tissue healing where fibronectin fragments stimulates the clearance of damaged tissue by resident cells that can actively remodel the ECM through contractile forces [9].

Under physiological conditions, ECM remodelling is normally self-limiting. Pathological remodelling arises when the balance between matrix deposition, crosslinking and degradation is disrupted as seen in diseases such as cancer and fibrosis which are thought to be "ECM-driven diseases". In both these diseases, excessive ECM deposition and remodelling lead to increased tissue stiffness, and abnormal biochemical signalling, leading to organ failure[25] (Figure 2). In contrast, excessive ECM degradation by abnormally high MMP levels result in cartilage destruction and cardiomyopathies[26, 27]. Since physiological and pathological ECM remodelling utilizes overlapping pathways, it is difficult to target without widespread systemic effects. It is therefore important to gain an understanding of the molecular control of ECM dynamics and the mechanisms that distinguish physiological and pathological remodelling

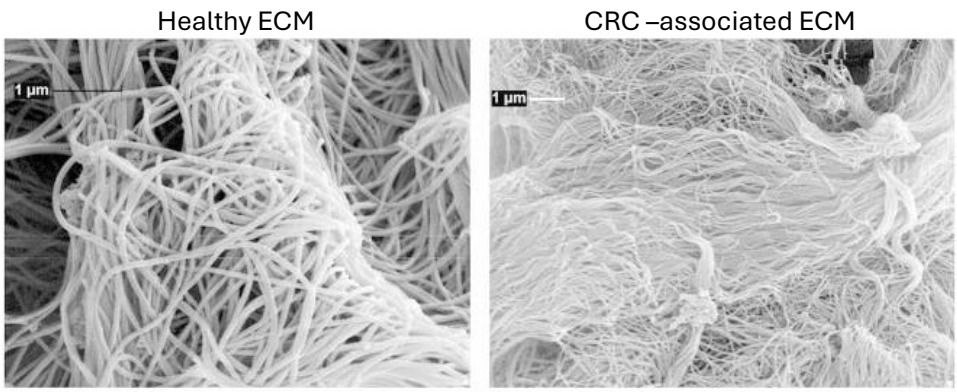


Figure 2. Dysregulation of the ECM is a hallmark of cancer.
Images show ECMs of the submucosa from healthy colon (left) and colorectal carcinoma (right) from the same patient. ECM from healthy submucosa showed thick fibres that formed random networks while ECM from the colorectal carcinoma displayed thinner and highly linearised ECM fibres. Image adapted from Nebuloni, M. et al. Insight On Colorectal Carcinoma Infiltration by Studying Perilesional Extracellular Matrix. Sci. Rep. 6, 22522; doi: 10.1038/srep22522 (2016). Distributed under the terms of the Creative Commons CC BY license (http://creativecommons.org/licenses/by/4.0/)

Fibroblasts

Fibroblasts are mesenchymal cells that have important roles in the development, maintenance and repair of connective tissues and their ECMs throughout the body. Morphologically, they are long spindle shaped cells with elongated nuclei. They lack unique biomarkers not shared among other cell types, making defining fibroblasts difficult. As a result, a combination of morphology, location and absence of lineage markers for leukocytes, epithelial cells and endothelial cells is used to define them[28, 29].

Fibroblast activation

In healthy tissues, fibroblasts are normally quiescent. In response to changes in their microenvironment, they can transition into activated states characterised by increased ECM production and remodelling activity. This is especially seen during wound healing[30] (Figure 3). At the site of the wound, immune cells secrete a number of cytokines including TGF-β and fibroblast growth factor. In response to these cytokine gradients, fibroblasts begin to proliferate and migrate to the wound. There, in addition to elevated cytokine levels, alterations in the mechanical properties of the surrounding disrupted ECM stimulate fibroblast activation into matrix fibroblasts. These cells secrete ECM components and ECM remodelling enzymes and replace the fibrin rich ECM with collagen, increasing the matrix stiffness. The increase in matrix stiffness further drives fibroblast activation into contractile proto-myofibroblasts that develop actin stress fibres. As these cells generate stronger contractile forces, they further stiffen the ECM, creating a positive feedback loop that promotes full differentiation into myofibroblasts. This transition is marked by the expression of alpha-smooth muscle actin (αSMA) which is incorporated into stress fibres, creating highly contractile cells. Concurrently, fibroblasts shift from a predominantly secretory phenotype to a contractile phenotype, enabling efficient wound contraction and complete the healing process[30, 31].

Following wound healing, myofibroblasts are cleared through either deactivation or apoptosis, displaying transient activation. However, during chronic injury and inflammation, the clearance of myofibroblasts may be hindered and they instead persist at the wound site, promoting excessive ECM remodelling and scarring as seen in fibrosis[29, 30, 32]. It is still unclear how a subset of fibroblasts can remain persistently activated as myofibroblasts.

The persistence of fibrotic myofibroblasts raises the possibility that fibroblasts found within the TME, called cancer associated fibroblasts (CAFs), may likewise adopt persistent phenotypes that contribute to CAF-mediated tumour protection and progression. Consequently, identifying the mechanisms responsible for fibroblast persistence is crucial to understanding and targeting these diseases.

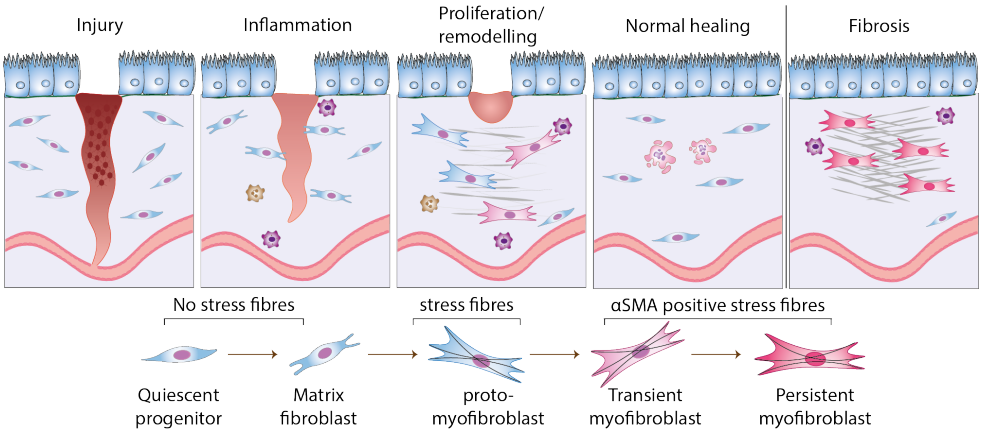


Figure 3. Normal tissue repair and fibroblast activation.
Fibroblasts are activated from a quiescent phase to proliferate and migrate to the site of the injury. There, the fibrin rich ECM is replaced by fibrous collagen produced by non-contractile matrix fibroblasts. The resulting denser ECM stimulates the formation of actin stress fibres and the appearance of contractile 'proto-myofibroblasts'. These cells produce even stiffer ECM which stimulate the expression and incorporation of αSMA into stress fibres, a hallmark of the highly contractile myofibroblasts. Disruption in myofibroblast clearance during normal healing can lead to myofibroblast persistence, resulting in fibrosis and scarring. Image created with Adobe Illustrator, adapted from [30].

Origin of myofibroblasts

How myofibroblasts originate is an area of ongoing study. While they have been shown to arise from resident fibroblasts, they have also been shown to arise from epithelial cells through epithelial-to-mesenchymal transition (EMT), endothelial cells through endothelial-to-mesenchymal transition (EndMT), pericytes, stellate cells and bone marrow derived mesenchymal stem cells. This plasticity adds to the complexity of targeting myofibroblasts therapeutically[29, 33].

Cancer associated fibroblasts

CAFs are currently identified as mesenchymal cells located in a tumour, lacking mutations found in cancer cells, with elongated morphologies and negative for lineage markers of leukocyte, epithelial and endothelial cells[34]. They form a major part of the tumour stroma and through functions similar to fibrotic fibroblasts, support tumour survival, metastasis and immune evasion. By promoting angiogenesis, aberrant ECM remodelling and secreting growth factors, they are known to create tumour supportive ECMs that facilitate stromal-epithelial interactions[34-36].

Various studies on CAFs have shown that altering stromal cells can be enough to influence cancer cell behaviour and tumour progression. In one such study, normal fibroblasts and CAFs isolated from patients were co-injected into mice with prostate

cancer cells. The group that received CAFs had significant tumour growth compared to the group that received normal fibroblasts[37]. Similarly, selective depletion of CAFs or CAF receptors such as Endo180 has been shown to inhibit tumour growth and metastasis[38, 39]. These findings highlight the critical role of CAFs in cancer progression.

CAF subtypes and functions

Similar to myofibroblasts, the origin of CAFs is unclear. In addition to resident fibroblasts, they are also thought to originate from a number of precursors resulting in CAFs being a heterogeneous population (Figure 4). Many attempts have been made to classify them into different subtypes based on their functions and single-cell RNA sequencing with the most widely recognized subtypes being myofibroblastic CAFs (**myCAFs**) and inflammatory CAFs (**iCAFs**).

myCAFs are located close to the cancer cells where they are exposed to high levels of TGF-β. Signalling through TGF-β can promote the expression of SMA, driving contractile ECM remodelling phenotypes as discussed earlier. They produce stiff ECMs that activate other stromal cells and act as a physical barrier to drug delivery and immune cell infiltration[40, 41]. However, emerging evidence suggests that this barrier function may have tumour restraining roles such as structural containment of tumour growth[42, 43].

In contrast, iCAFs are located further away from the cancer cells and predominantly secrete immunosuppressive signals. Pro-inflammatory signals such as IL-1 secreted by tumour cells activate the JAK/STAT pathway in CAFs which drives the secretion of inflammatory mediators such as IL-6 that activate other CAFs and various other cytokines to convert monocytes to tumour-associated macrophages[44-46]. Other immune cells such as T-cells can be pushed to differentiate into tumour supportive Treg cells by iCAFs [47].

Other CAF subtypes include antigen presenting CAFs (**apCAFs**) that express MHC class II molecules. They interact with $CD4^+$ T cells, rendering them non-functional through anergy or promoting their differentiation into Tregs[48]. Metabolic CAFs (**metCAFs**) display high oxidative phosphorylation and lipid metabolism to supply cancer cells with metabolic intermediates[49]. Vascular CAFs (**vCAFs**) support angiogenesis[50] while tumour-restraining CAFs (**rCAFs**) can paradoxically exhibit tumour suppressive features. It was found that in early stages of cancer, normal fibroblasts had cancer growth inhibiting roles. rCAFs seem to exhibit some of the same markers as normal fibroblasts such as Meflin which inhibits collagen remodelling and the formation of dense collagen structures[51].

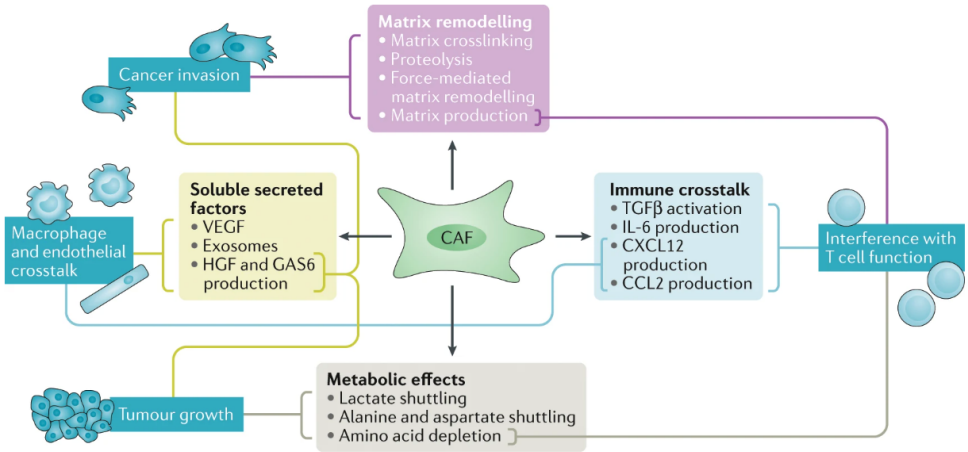


Figure 4. CAFs have diverse subtypes and functions in the tumour microenvironment.
CAFs support tumour survival through abnormal matrix remodelling which promotes cancer cell invasion, secrete soluble factors such as growth factors, supress the immune system and provide metabolic intermediates. Image from Sahai, E.*et al.* A framework for advancing our understanding of cancer-associated fibroblasts. *Nat Rev Cancer* **20**, 174–186 (2020). https://doi.org/10.1038/s41568-019-0238-1. Distributed under the terms of the Creative Commons CC BY license (http://creativecommons.org/licenses/by/4.0/)

Mechanical cues in the tumour microenvironment

The tumour microenvironment is a dense ecosystem of cellular and non-cellular components with distinct mechanical properties that differ from normal healthy tissues (Figure 5). It can be broadly divided into matrix mechanics, solid and fluid stresses. This thesis focuses on the effects of matrix mechanics which will be the focus of this section.

Excessive ECM deposition and reorganization

One of the main drivers of ECM stiffening in tumours is the overproduction of ECM components. CAFs can produce excessive amounts of collagen I and III, creating a dense and fibrous stroma. This accumulation increases matrix density and reduces porosity, making the tissue more rigid[52]. Importantly, matrix stiffening is not only a consequence of ECM accumulation but also a driver of further remodelling as

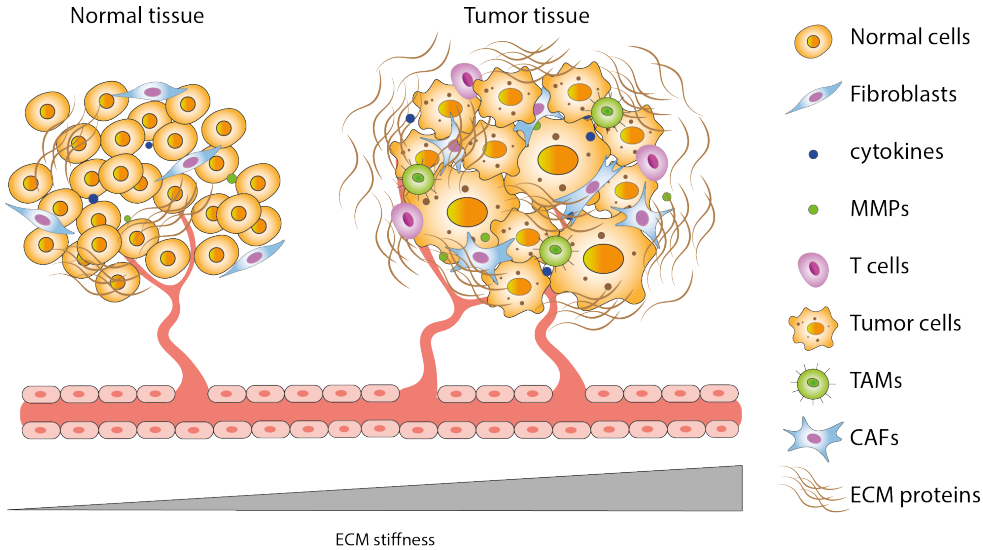


Figure 5: Normal vs Tumour tissue
Tumours are a dense ecosystem of cellular and non-cellular components. They undergo excessive remodelling by CAFs, tumour cells and immune cells to increase tissue stiffness and create microenvironments favourable for tumour growth and survival. Increase in ECM stiffness activates CAFs, creating a positive feedback loop between CAF activation and tumour stiffness, resulting in the development of a progressively fibrotic and rigid microenvironment. Image created with Adobe Illustrator.

mechanical signalling can stimulate CAFs to produce more structural proteins and downregulate MMPs production to prevent ECM degradation[53, 54]. Consequently, matrix stiffness mediated activation of ROCK signalling pathways further promotes collagen, fibronectin and laminin production that alter matrix architecture[55, 56]. Elevated fibronectin and tenascin-C levels promote the alignment and bundling of collagen fibres into ordered, tightly packed networks capable of transmitting greater tensile forces[57, 58]. Concurrently, increased levels of Glycosaminoglycans (GAGs) and hyaluronic acid enhance tissue hydration, contributing to matrix stiffening by increasing interstitial fluid pressure[59].

Growth factors such as TGF-β, PDGFRα, and TNFα sustain these processes by enhancing CAF activation and matrix deposition. TGF-β acts as a central regulator of ECM remodelling by modulating the expression of a number of ECM related genes by stimulating the nuclear translocation of transcription factors such as YAP/TAZ, SMAD2/3, Twist1 and Ets-1[60-62]. In parallel PDGFRα signalling promotes matrix accumulation by stimulating hyaluronic acid production[63] while TNFα has been shown to increase the deposition of proteoglycans[64].

In addition to a highly ordered ECM, collagen crosslinking further increases matrix stiffness. Enzymes such as LOX, LOXL and Lysyl Hydroxylase 2 overexpressed by

tumour cells and CAFs crosslink collagen and increase the tensile strength of collagen fibres, making them resistant to degradation[53].

Increased Cell Contractility

Activated CAFs and tumour cells can exhibit heightened contractility where forces generated by actomyosin networks are transmitted to the ECM through adhesion complexes and exert pulling forces on bound ECM components, aligning and bundling them, thereby increasing ECM stiffness overtime. Many ECM components also exhibit strain-stiffening where constant deformation results in increasing stiffness[65].

High ECM stiffness, biochemical signals from the tumour and activated CAFs can further activate other fibroblasts and cell types into CAFs, creating a positive feedback loop between CAF activation and ECM stiffness[34]. This loop leads to the development of a progressively fibrotic and rigid microenvironment.

Physical Factors (Hypoxia and Pressure)

Proliferation of tumour cells and excessive ECM deposition in confined spaces can increase physical compression and solid stress. This increase in tissue pressure compacts the ECM and reduces spacing between ECM fibres, further increasing ECM stiffness.

Further, solid tumours are often associated with poor blood and lymphatic drainage, resulting in fluid retention and high interstitial fluid pressure. Constricted blood vessels and vascular collapse in tumours can also give rise to hypoxic environments that stimulate CAF activity. In response to hypoxia, CAFs express transcription factors such as HIF-1α (Hypoxia-inducible factor-1 alpha) that promotes conversion of fibroblasts into CAFs[66].

Consequently, tumour ECM stiffening emerges from the combined effects of matrix deposition, fibre organization and mechanosensitive feedback signalling which together establish a self-reinforcing fibrotic environment.

Mechanical signalling: Linking force to function

Mechanical signals from the ECM such as stiffness and composition are sensed through specialized structures called integrins and FAs. These structures can anchor cells to the ECM and, through a process known as mechanotransduction, translate mechanical cues from the ECM into intracellular signals. These signals regulate cell behaviours such as migration, proliferation and differentiation, allowing cells to coordinate diverse processes such as development, wound healing and immune responses and adapt to changing environments[67, 68].

Integrins receptors: Gateways to the ECM

Integrins are the main cell adhesion receptors that connect the cells' cytoskeleton to the ECM. They are heterodimeric, composed of non-covalently bound α and β glycoprotein subunits. In mammals, there are 18 α and 8 β subunits that combine to form 24 heterodimeric receptors. Structurally, integrins display an extracellular domain, a hydrophobic transmembrane domain and a cytoplasmic domain (Figure 6).

The extracellular domain consists of an elongated stalk and a globular headpiece that binds to extracellular ligands such as fibronectin. In the globular headpiece, all β subunits possess an "I-like domain" involved in ligand binding while half the α subunits have "I-domains". Ligand recognition by the extracellular domains depends on short peptide motifs found within ECM proteins such as the RGD (Arg-Gly-Asp) motif found in vitronectin and fibronectin.

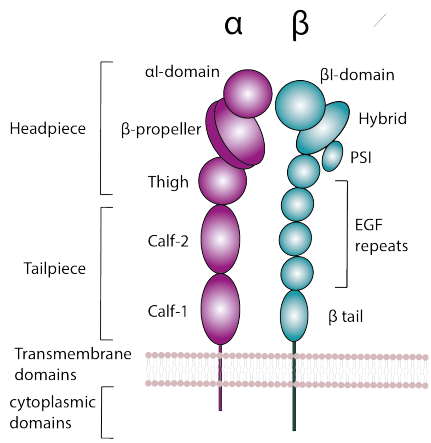


Figure 6: Integrin structure

Integrin αβ heterodimers have extracellular domains that bind to ECM ligands, a transmembrane domain and cytoplasmic domains that transmits ECM signals to the cell interior. Image created with Adobe Illustrator.

The integrin cytoplasmic domains are responsible for intracellular signalling, particularly at the β tail. Most β subunits have a "NPxY" motif which binds a number of signalling molecules and recruit adaptor proteins that bind actin filaments, creating integrin-actin connections[69-71].

Integrin activation

Integrin activation is mediated through conformational changes in their structures from bent inactive states to extended active states. In their inactive states, they are restrained through interactions between their cytoplasmic domains and the headpiece is folded towards the membrane, preventing ligand binding and intracellular signalling. They can be primed to an intermediate state and activated bidirectionally, from the outside-in, through external ligand binding, or inside-out, through intracellular molecule binding[72] (Figure 7).

Inside-out signalling requires the binding of adaptor proteins to the β cytoplasmic tails. One such adaptor protein is talin, normally distributed in the cytosol in its autoinhibited form. Upon stimulation, talin is recruited and activated at the membrane by PIP2. Rap1, a small GTPase downstream of priming, is thought to bind talin and target it to the β cytoplasmic tail, enabling talin binding to NPxY motifs. Talin binding can induce the separation of the integrin cytoplasmic domains, leading to integrin activation and clustering. Other important effectors binding at the β tail are the Kindlin family that bind to distal NxxY motifs[73, 74].

During outside-in signalling, integrins are activated through external ligand binding which depends on integrin affinity and avidity. Affinity or the 'binding strength' is increased through conformational changes to high affinity states. This is mediated by the binding of small peptide fragments at the I-domains or β propeller domain of the α subunits and I-like domain on β subunits that can help strengthen binding to larger ligands. New binding regions called 'ligand induced binding sites' are exposed during these conformational changes to further enhance adhesion[75, 76].

Integrin avidity, which is the overall strength of adhesion between a cell and its environment is enhanced through integrin clustering. At the plasma membrane, clustering is favoured in regions where the membrane and ligands are in close proximity and have pre-existing contacts. This creates a 'kinetic trap', concentrating integrins into clusters[77, 78].

Integrin engagement, conformational changes and clustering trigger the recruitment and development of complex integrin-associated adhesion complexes (IAC) such as focal adhesions, allowing bidirectional transmission of forces and downstream signalling pathways that are discussed in the coming sections.

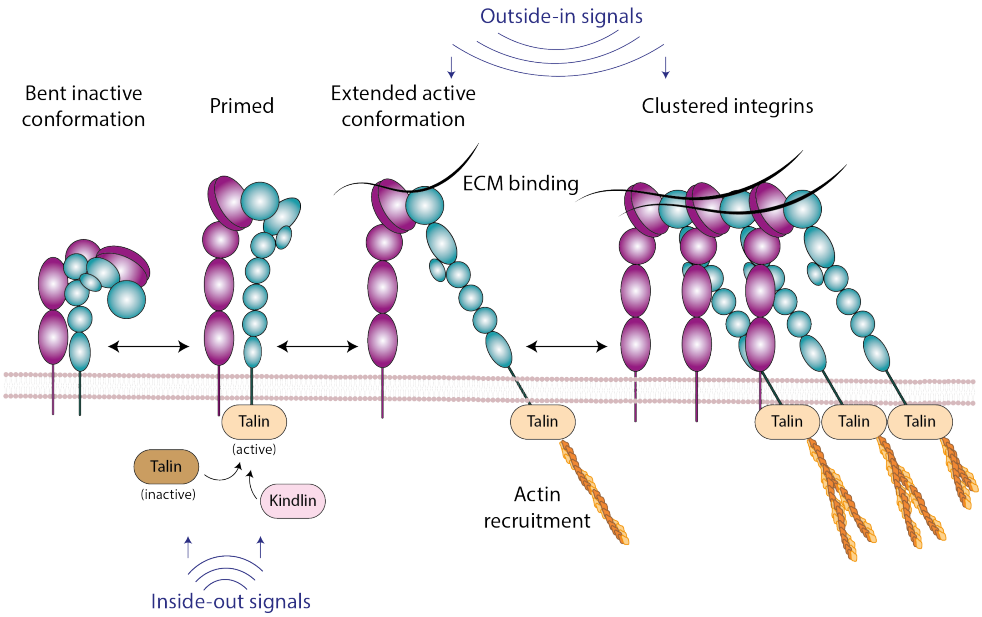


Figure 7: Integrin conformational states regulate their activity.
During activation, integrins undergo conformational changes from bent, inactive conformation to extended, active conformations through inside out and outside in signalling. These structural transitions regulate ligand binding, receptor affinity and the initiation of downstream signalling pathways. Image created with Adobe Illustrator, adapted from [79].

Focal adhesions

FAs are large, dynamic multilayered protein complexes that connect ECM bound integrins with the cell's actin cytoskeleton. They are the most prominent type of IACs and serve as structural anchors and signalling hubs. Their molecular composition, organization and downstream signalling vary greatly based on their location, function and type of biochemical or mechanical signals they receive from their environment. However, all FAs have integrin receptors on one end and actin stress fibres at the other. They are largely organized into 3 layers: the bottom integrin signalling layer, middle force transduction layer and top actin regulatory layer[80, 81] (Figure 8).

Within the integrin signalling layer, integrins function as the primary ECM receptors with scaffolding proteins such as paxillin and signalling molecules such as Focal adhesion kinase (FAK) and ILK assemble signalling complexes at the adhesion site[81, 82]. Although paxillin has no enzymatic activity itself, it can bind to integrins and stabilize early adhesions through its large number of binding partners such as other structural proteins within FAs, facilitating FA protein interactions, and signalling proteins such as FAK involved in downstream signalling pathways. Upon

recruitment, FAK is activated through autophosphorylation on Tyr397 which acts as a high affinity binding site for Src family kinases. Src in turn phosphorylates FAK at Tyr576 and Tyr 577 in the kinase domain, and at Tyr 861 and Tyr 925 at the C-terminal region for maximal catalytic activity. Through its association with the Src kinase family, the FAK-Src complex is a critical player in downstream signalling[83].

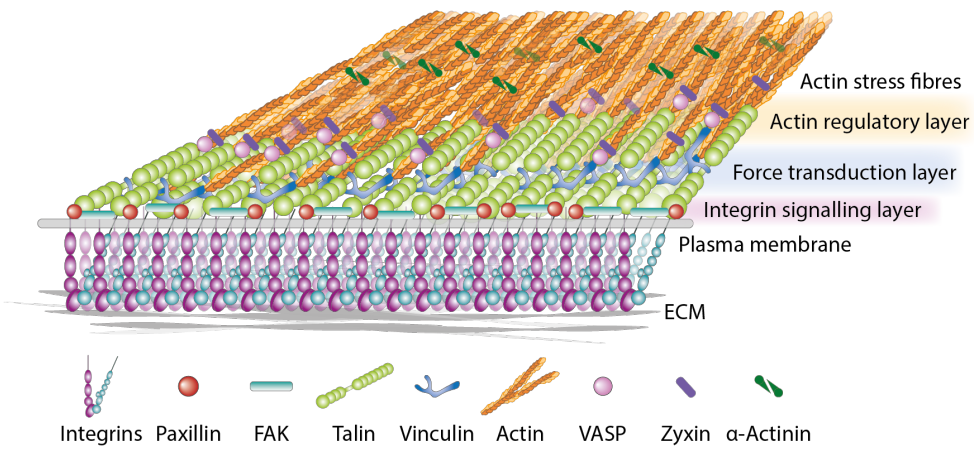


Figure 8: Structure and organization of focal adhesions
FAs are composed of hundreds of proteins organized into distinct multilayered structures. At the plasma membrane, integrins bind to the ECM on one side and to FA proteins on the other. Mechanical forces are transmitted bidirectionally between ECM bound integrins and actin bound FA proteins through the force transduction layer. The actin regulatory layer regulates actin polymerization and cytoskeletal dynamics, thereby contributing to FA assembly, remodelling and force transmission. Image created with Adobe Illustrator, adapted from [84].

The force transduction layer converts these signalling events into mechanical outputs. Talin and vinculin form the principal physical connection between integrins and actin. Upon binding to integrins and actin, talin transmits cell- generated forces to the ECM and ECM-generated forces to the cell. The application of force across talin releases it from its autoinhibitory conformation to its extended active conformation, exposing cryptic binding sites for vinculin among others. Vinculin is a scaffolding protein recruited by paxillin through paxillin phosphorylation by FAK. It binds to talin through its head domain and to proteins in the actin regulatory layer through its tail domain, reinforcing the adhesion[85, 86].

At the actin regulatory layer, VASP and zyxin are recruited together to coordinate actin remodelling. Zyxin binds actin and actin crosslinking proteins α-actinin. VASP, through its EVH1 domain, binds zyxin, vinculin and Guanine exchange factors (GEFs) through which they regulate actin polymerization[81].

Together, these layers function as an integrated mechanosensory system through which cells can sense the ECM and dynamically adapt to their environment.

FA maturation and mechanosensor activity

FAs undergo a maturation process wherein they grow from small early adhesions called "nascent adhesions" at the cells lamellipodia. These are transient dot-like structures that persist for ~60sec. They are generated through myosin II independent actin polymerization and display active signalling through phosphorylation of proteins such as paxillin, vinculin, FAK and Src. Nascent adhesions disassemble during active protrusion when actin is depolymerized but otherwise mature into "focal complexes" at the boundary of the lamellum. These are larger structures associated with myosin II activity and are characterized by the recruitment of vinculin. Focal complexes can continue to mature into FAs which actively participate in ECM remodelling, force transmission and signalling and are rich in vinculin, paxillin, FAK, and talin. Fibrillar adhesions can evolve from mature FAs, forming large structures found centrally that can persist for several hours. They are characterised by high concentrations of Tensin and low levels of vinculin and paxillin[87].

Regardless of the nature of the stimulating signal, FA maturation depends on the magnitude of mechanical forces acting across them. Physical cues from the environment such as ECM stiffness, composition and ligand density are converted into forces when cells try to pull or push on ECM fibres. On stiff ECMs, cells experience greater resistance to deformation. This resistance provides a mechanical anchor against which actomyosin contractility can generate higher intracellular tension, favouring the recruitment of FA components and FA maturation[68, 88].

Depending on the magnitude and direction of these forces, mechanosensory proteins such as integrins, talin and vinculin, undergo specific force driven changes in conformation, function and binding partner interactions during FA maturation which are discussed in the following sections.

Mechanosensor responses to magnitude of force

Integrins exhibit catch bond behaviours where forces generated through pulling on bound ligands can increase the bond lifetime. This behaviour contrasts with the more common slip bonds where the bond weakens upon application of force. This is due to the conformational properties of integrins. In the absence of force, integrins may bind their ligands weakly and transiently. When tensile force is applied through either actomyosin contractility or external mechanical stress, it promotes the alignment of binding domains between the integrin head piece and the ligand such as extracellular fibronectin or intracellular adhesion molecules, enhancing non-covalent interactions. On soft ECMs which are easily deformable, "pulling forces" generated at the integrin-ligand interface are low, resulting in failed alignment and prevent catch bond formations. Optimal loading rates with magnitudes of 10-30pN generated on highly resistant stiff ECMs can pull integrins into their extended active conformations, facilitating outside-in signalling[89, 90].

Structurally, talin consists of a globular head domain that binds to integrins, and a rod domain composed of multiple α-helical bundles that are folded in resting states. When mechanical force is applied across talin through either force transduction from stable ECM-integrin links during outside-in signalling or myosin-based contractility during inside-out signalling, the talin rod begins to unfold. The unfolding occurs sequentially in a stepwise manner with each domain requiring a specific threshold of force, enabling talin to function as a finely tuned force sensor[85, 91].

Talin unfolding exposes cryptic binding sites for vinculin, whose binding stabilizes talin unfolding and adhesion maturation, amplifying the cells mechanical response. Binding of vinculin to talin activates vinculin by releasing it from its autoinhibited form. Activated talin and vinculin can then form catch bonds with actin filaments, strengthening their links to actin under tension and stabilizing their active conformations when subjected to a certain range of force[92, 93] (Figure 9).

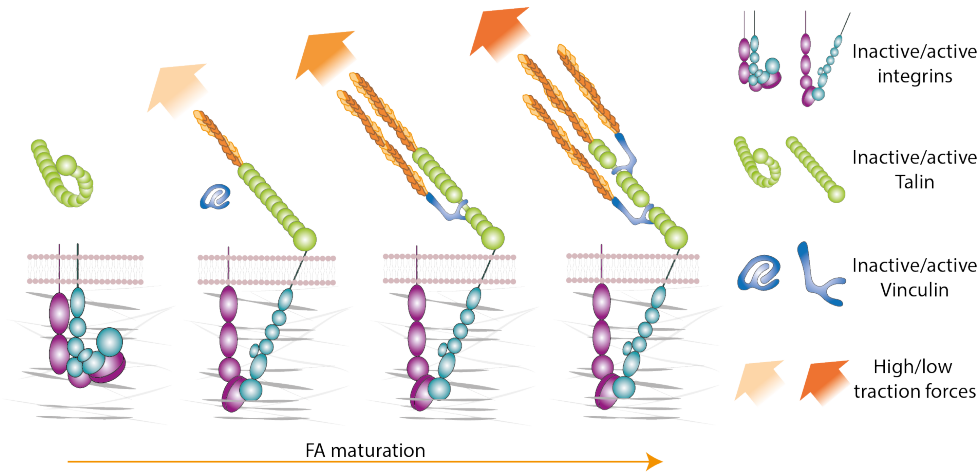


Figure 9: Mechanosensors during FA maturation
Mechanosensory proteins undergo force driven changes in conformation, function and binding partner interactions during FA maturation. Image created with Adobe Illustrator.

Mechanosensor responses to direction of force

Apart from magnitude, the direction of force is also an important factor in mechanosensor activity. Mechanosensors are proposed to be distributed in an anisotropic manner in the cell and only those oriented parallel to the direction of force undergo greater mechanical activation than those oriented perpendicular. This directional sensitivity allows cells to extract spatial information from their environment and respond in a coordinated, polarized manner.

For example, talin unfolds when tensile force is applied along the long axis of the talin molecule. The catch bonds between the actin binding sites of talin and vinculin with actin form only when force is applied towards the filaments pointed end[92, 94]. Similarly, vinculin binding to talin is promoted when force is applied parallel to the binding site[95]. Upon binding, talin and vinculin can further re-orient themselves along the axis of force[96]. Integrins have also been shown to be aligned by retrograde actin forces, showing directional polarization during cell migration[97].

At the level of FAs, directionality of force influences their growth and orientation. Integrins, FAs and stress fibres are aligned along the direction of applied tension. This alignment enhances force transmission efficiency and reinforces adhesion structures. As a result, cells can become polarized with defined rear and front, enabling directional migration[98].

The 4 types of cytoskeletal networks at FAs

Although actin is the main cytoskeletal component associated with FAs, FAs are not exclusively linked to actin filaments. They are dynamic hubs that integrate multiple cytoskeletal systems to coordinate cell adhesion and signalling. In addition to actin, microtubules, intermediate filaments and septins also interact with and contribute to FA regulation.

Actin

As previously discussed, actin filaments are the most directly linked cytoskeletal component to FAs and are the primary force generating network. The amount of force generated depends on the cellular location of the adhesions and the type of actin structures assembled. For example, in actively migrating cells, nascent adhesions and focal complexes are associated with branched actin and stress fibres with low myosin II activity at the leading edge where fewer forces are generated through actin polymerization and actomyosin contractility. Conversely, FAs associated with actin stress fibres at cell protrusions experience forces generated by myosin IIA activity that stabilizes them. At the cell's central and rear, myosin IIB drives adhesion maturation[99-101].

Actin cytoskeleton dynamics are governed by regulatory proteins that control filament nucleation, elongation, branching and disassembly. The most central of these are the Arp2/3 complex and formin family. The Arp2/3 complex nucleates branched actin filament networks and requires nucleation promoting factors such as members of the WASP/WAVE family which deliver actin monomers and induce conformational changes that mimics a preformed actin dimer. Once activated, Arp2/3 binds to the sides of the pre-existing "mother" filament and nucleates a new "daughter" filament at a 70° angle resulting in a branched network as in lamellipodia[102].

Formins are a large family of proteins that promote the elongation of unbranched linear actin filaments such as stress fibres and filopodia. They are characterized by the presence of formin homology domains FH1 and FH2. The FH2 domain forms a dimeric ring that associates with the barbed end of a growing actin filament, protecting it from capping proteins and allowing the addition of new actin monomers. The FH1 domain recruits profilin bound actin monomers, accelerating filament elongation[103].

Microtubules

Microtubules are composed of α- and β-tubulin heterodimers arranged in a cylindrical manner. They dynamically target FAs which promotes their turnover, allowing the cell rear to detach during cell migration. Depolymerization of microtubules releases GEF-H1 that activates RHO, increasing actomyosin contractility and promoting FA growth and maturation.

Microtubules also act as tracks for transporting proteins to and from adhesion sites. They can deliver and recycle integrins during FA assembly and disassembly and transport actin regulators, intermediate filaments and enzymes such as matrix metalloproteases (MMPs) to remodel the ECM[104].

Intermediate filaments

Intermediate filaments (IFs) are rope like structures that act as stress absorbers. Vimentin is the primary IF protein in fibroblast FAs. They bind to integrins and actin crosslinkers such as filamin A and plectin to initiate IF growth and polymerization which in turn inhibits FA disassembly, promoting fibrillar adhesion[105].

Septins

Septins are GTP-binding proteins that have recently emerged as important regulators of FA dynamics and mechanotransduction. The 13 human septins are classified into 4 groups (SEPT2, SEPT3, SEPT6, SEPT7) based on sequence homology within their C- and N-terminal domains and GTPase activity. Monomers from each group self-assemble into heterooligomers and higher order structures such as filaments and rings, enabling septins to function as dynamic scaffolds involved in cytokinesis and vesicle trafficking[106].

Within the FA machinery, septin filaments associate with actin stress fibres and localize near adhesions sites where they coordinate the recruitment and organization of cytoskeletal and signalling proteins required for FA maturation. Although several septins have been implicated in these processes, SEPT6, SEPT7 and SEPT9 appear to have prominent roles in mechanosensitive signalling[107]. SEPT6 and SEPT9 respond to changes in ECM stiffness by regulating the nuclear transportation of adaptor proteins[108, 109], whereas SEPT7 functions as the core structural component

of septin complexes. Unlike many other septins, SEPT7 cannot be replaced by another septin protein.

The importance of septins in force dependent adhesion is evident in CAFs where an increase in SEPT7 and SEPT2 containing filaments promotes FA maturation and downstream signalling on stiff ECMs Further, depletion of SEPT7 disrupts septin filament integrity, resulting in smaller, less stable FAs and accelerated FA turnover. These findings identify septins as key regulators linking ECM mechanics to FA maturation and mechanotransduction[110 111].

Mechanotransduction: Translating mechanical signals

Upon integrin engagement, a cascade of signalling pathways is activated at FAs. Several of these major mechanotransduction pathways have been identified involving both cytoskeletal structures and biochemical factors that work in coordination (Figure 10).

FAK-Src signalling pathways: The FAK-Src complex initiates multiple signalling cascades including activation of Rho family GTPases (controls cytoskeletal dynamics and cell polarity), MAPK/ERK pathway (promotes proliferation and migration) and PI3K-AKT pathway (survival and metabolism) that can drive fibroblast activation. Dysregulation of FAK-Src signalling is associated with increased cell motility, invasion and resistance to apoptosis in various cancers[112].

Rho family GTPases: The Rho family of GTPases (RhoA, Rac1 and Cdc42) are key molecular switches that regulate the organization and dynamics of the actin cytoskeleton. RhoA promotes actomyosin contractility and FA maturation by activating ROCK which phosphorylates myosin light chain (MLC), enhancing stress fibre formation[113]. RhoA mediated actin polymerization releases MRTF-A (myocardin-related transcription factor-A) that can move to the nucleus to turn on pro-fibrotic genes such as αSMA[114].

MAPK/ERK pathway: Phosphorylation of adhesion proteins such as paxillin and Shc by FAK-Src leads to the activation of small GTPases RAS which triggers a kinase cascade that ultimately activates mitogen activated protein kinases (MAPK)/ERK kinase. Activated ERK can translocate to the nucleus to phosphorylate transcription factors and regulate gene expression related to cell cycle progression, migration and myofibroblast differentiation[115].

PI3K-AKT pathway: FAK-Src activity can recruit adaptor proteins that bind and activate phosphoinositide 3-kinase (PI3K) which phosphorylates membrane lipids that serve as a docking site for proteins with pleckstrin homology domains such as Akt. Once localized to the membrane, Akt is phosphorylated and activated by PDK1 and mTORC2.

Akt promotes cell survival by inhibiting pro-apoptotic proteins (e.g. BAD) and modulates actin dynamics, FA turnover and integrin recycling. It also intersects with Rho GTPase signalling to fine tune cytoskeletal tension and cell polarity[116, 117].

YAP/TAZ: YAP (Yes-associated protein) and TAZ (transcriptional co-activator with PDZ-binding motif) are mechanosensitive transcriptional regulators. Mechanically, YAP/TAZ are retained in the cytoplasm when cytoskeletal tension is low (e.g. when cells are on soft substrates). High tensions sustained by mature FAs disrupts the cytoplasmic retention of YAP/TAZ, allowing their accumulation in the nucleus. In the nucleus, YAP/TAZ bind to TEAD transcription factors, modulating the expression of genes involved in proliferation, survival and matrix remodelling[118].

P130Cas-Crk-DOCK complex: P130Cas (Crk-associated substrate) is an adaptor protein recruited to the FAs upon integrin engagement and FAK-Src signalling. Src family kinases phosphorylate tyrosine residues in p130Cas, creating binding sites for Crk, another adaptor protein. Crk in turn recruits DOCK180 (dedicator of cytokinesis 1), a GEF that activates Rac that drives actin polymerization and integrin recruitment[119].

ILK-PINCH-Parvin complex (IPP complex): The ILK-PINCH-Parvin complex is a core structural and signalling module at FAs, critical for linking integrins to the actin cytoskeleton and regulating processes such as adhesion, spreading, migration and survival. Integrin linked kinase (ILK) binds to the cytoplasmic tail of β integrins, anchoring the complex at FAs with scaffolding functions. PINCH (particularly interesting new cysteine-histidine rich protein) is an adaptor protein that binds ILK at ankyrin repeats and connects to other regulatory proteins. Parvin interacts with ILK and F-actin, providing direct physical connection between integrin and the actin cytoskeleton.

The IPP complex stabilizes FAs and contributes to actin filament organization and regulates signalling pathways such as PI3-Akt pathway. Dysregulation of the ILK-PINCH-Parvin complex compromises cell adhesion and cytoskeletal architecture, highlighting its role in maintaining tissue integrity[120].

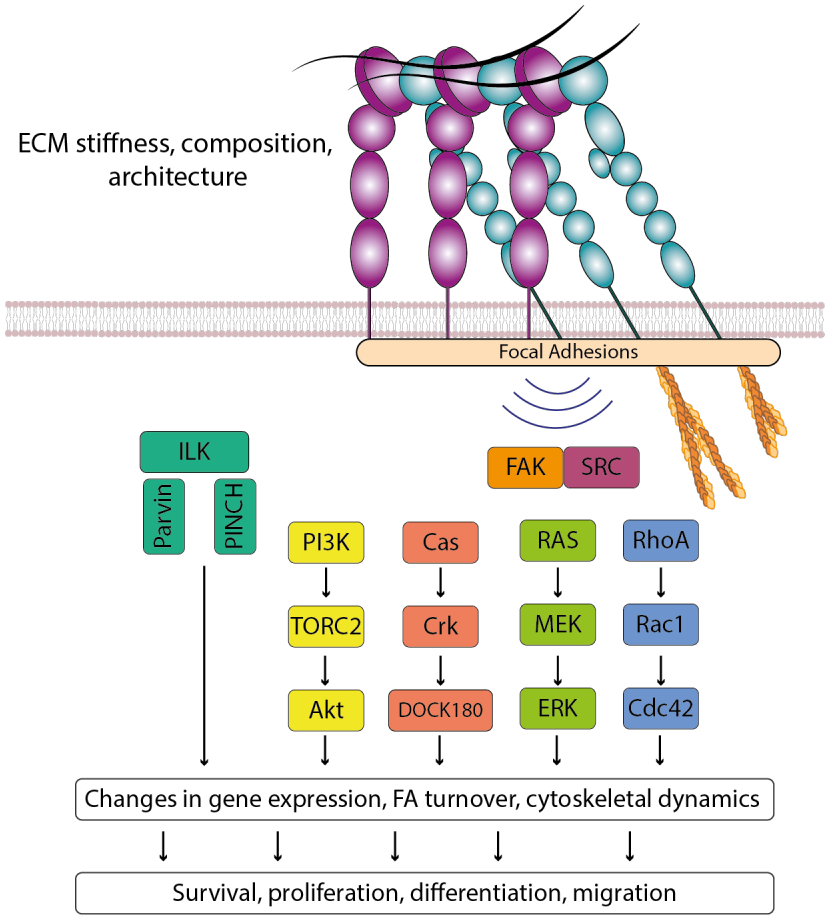


Figure 10: FA signalling
Mechanotransduction pathways involve both cytoskeletal structures and biochemical factors that regulate changes in FA turnover, cytoskeletal dynamics and gene expression that ultimately drive cell survival, proliferation, differentiation and migration. Image created with Adobe Illustrator.

The nucleus as a mechanosensor

Following ECM sensing, forces generated at FAs are propagated through the cytoskeleton and transmitted to the nucleus. This direct mechanical coupling has led to the growing appreciation of the nucleus as a mechanosensitive organelle that actively senses and responds to ECM derived cues. By undergoing force induced changes in its architecture, composition and mechanical properties, the nucleus plays a central role in translating physical cues into cellular responses. Mechanical forces regulate chromatin organization, gene expression, nuclear transport of transcription factors and nucleocytoplasmic transport, positioning the nucleus as a key mediator of cellular mechanotransduction [121] (Figure 11).

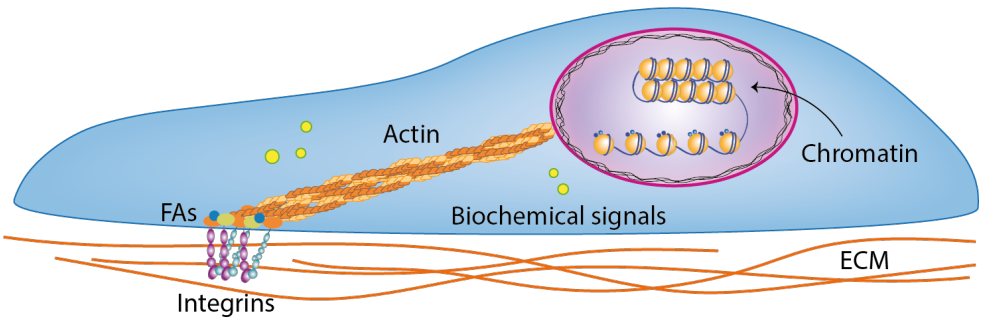


Figure 11: Cellular mechanotransduction
Forces generated at the cell-ECM surface at regions of FAs are sent to the nucleus via the cytoskeleton. These mechanical forces can deform the nucleus, altering gene expression patterns and cellular responses. Image created with Adobe Illustrator.

Mechanical coupling of nuclear components

The nucleus is a membrane bound organelle. At the outermost level is the nuclear envelope, a double membrane structure consisting of inner and outer membranes that separate the nucleoplasm from the rest of the cell. The outer membrane interacts with the actin cytoskeleton, and the inner membrane is supported by a network of proteins called the nuclear lamina. These connections on either side of the envelope are pivotal for force transmission to and into the nucleus[121, 122].

LINC complex

The nucleus is coupled to the cytoskeleton through the “linker of nucleoskeleton and cytoskeleton (LINC) complex” that spans the nuclear membrane on both sides. It consists of the SUN domain-containing proteins that span the inner membrane and KASH domain containing proteins, called Nesprins, that span the outer membrane. Within the space between the two membranes, SUN proteins form trimeric assemblies with their C-terminal domains engaging with conserved KASH

peptide motifs of nesprins, forming a continuous physical bridge across the nuclear envelope. Structural studies have shown that this SUN-KASH interaction forms highly stable force resistant interface capable of transmitting piconewton-scale forces across the nuclear envelope[123] (Figure 12).

On the cytoplasmic side, nesprins can have different binding partners depending on their isoforms. Nesprin 1 and 2 giant isoforms can bind to F-actin, Nesprin 3 binds intermediate filaments and Nesprin 3 and 4 bind to microtubule motors. On the nucleoplasmic side, SUN proteins interact with nuclear lamins and chromatin, creating a physical pathway through which forces are propagated into the nucleus to influence chromatin architecture and transcriptional activity[123, 124].

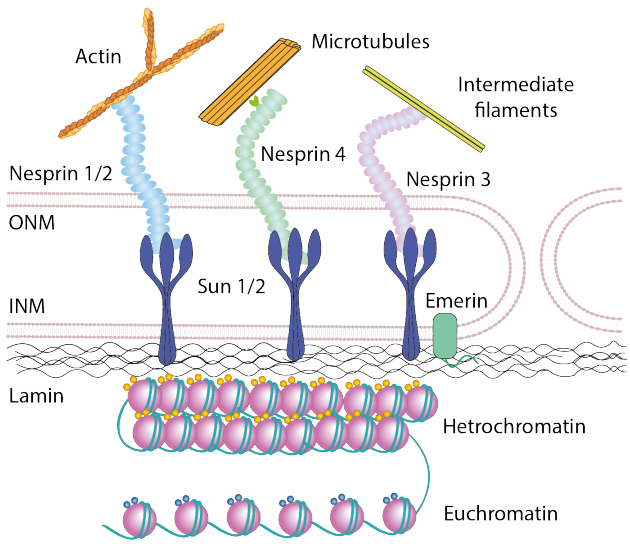


Figure 12: The nuclear envelope
The nuclear envelope is connected to cytoskeletal components at its outer membrane and to the nuclear lamina and chromatin at the inner side of the inner membrane. The LINC complex transverses the envelope, connecting the cells cytoskeleton to chromatin, creating ECM-chromatin links. Image created with Adobe Illustrator, adapted from [125].

Emerin

Emerin is a LEM domain protein primarily localized to the inner membrane with the majority of the protein facing the nucleoplasm. There, it interacts with nuclear lamins and transcriptional regulators, facilitating the transmition of forces from the cytoskeleton into the nucleus. Its N terminal LEM domain mediates binding to BAF (barrier to autointegration factor), a chromatin associated protein which promotes chromatin compaction and tethering at the nuclear envelope[126]. In addition to regulating nuclear actin dynamics, Emerin has also been shown to affect actin organization throughout the cell where loss of emerin disrupts the mechanical

coupling between the actin cytoskeleton and the nucleus, highlighting its essential role in nuclear mechanotransduction[127].

Lamins

Nuclear lamins are type V intermediate filaments and are major components of the nucleoskeleton. They form a mesh like network along the inner nuclear membrane and regulate nuclear stiffness and integrity. Lamin B is closest to the membrane and provides structural elasticity, allowing the nuclear envelope to endure deformation while Lamin A/C contribute to nuclear stiffness and can also be found in the nucleoplasm. The nuclear lamina is linked to the cell's cytoskeleton through the LINC complex on one side and directly to the chromatin on the other, thereby enabling force mediated gene expression changes[128, 129].

In response to mechanical stimuli, lamins rearrange themselves, undergo posttranslational modifications and alter their interactions with other molecules. In addition to their rearrangement, the ratios of lamin A/C to lamin B expression levels are also sensitive to mechanical cues. While Lamin B levels have been observed to remain relatively constant regardless of matrix stiffness, an increase in ECM stiffness results in an increase in lamin A levels. Conversely, soft matrices lead to lower lamin A expression, resulting in a more pliable nucleus. This scaling of nuclear stiffness with ECM stiffness is necessary to enable the nucleus to withstand environmental tensions[129, 130].

The nuclear lamina interacts with numerous transcription factors including YAP/TAZ. Under conditions of high ECM stiffness, lamin A/C accumulation promotes the nuclear retention of YAP/TAZ, facilitating their transcriptional activity[131]. Lamins are also involved in the formation of lamina associated domains (LADs) that cover a substantial fraction of the genome (30-40%) in mammalian cells. These domains are generally associated with transcriptionally repressed genes and are enriched in repressive epigenetic marks such as H3K27me3 which contributes to chromatin compaction and transcriptional silencing. This arrangement segregates repressed heterochromatin towards the lamina and active euchromatin towards the nuclear interior. However, recent studies have shown that certain "escaper genes" may undergo active transcription within LADS, suggesting that lamins are engaged in multiple types of gene expression regulation[132].

Chromatin

Chromatin directly contributes to nuclear mechanotransduction through its polymeric architecture and organization. It functions as a viscoelastic material and its response to force depends on the compaction state, crosslinking densities and interactions with nuclear proteins[133]. Similar to lamins, heterochromatin forms densely packed domains that increase nuclear stiffness and resistance to deformation. Perturbations that globally decompact chromatin reduce nuclear

stiffness even in the absence of changes to lamins, suggesting that chromatin itself is a load bearing structure[134].

Mechanical stresses transmitted across the nuclear envelope are distributed through chromatin fibres that form a percolating network spanning the nucleus. Polymer physics models describe this network as a crosslinked, confined polymer gel where force propagation is not uniform, but instead depends on local density variations and connectivity[135]. These forces can induce changes in histone modifications, chromatin reorganization and ATP dependent chromatin remodelling. Such responses can occur independently of the nucleoskeleton, indicating that chromatin is mechanosensitive and capable of converting physical cues into biochemical outputs[136, 137].

Nuclear pore complex

Mechanical forces transmitted from the cytoskeleton can deform the nuclear envelope and alter nuclear pore complex (NPC) geometry and diffusion, thus, providing a direct physical mechanism by which mechanical cues can regulate nuclear import and export without requiring changes in transport receptor activity. Certain mechanical stimuli can also induce alterations in the structure and transport kinetics of transcription factors such as YAP/TAZ and MRTF-A in ways that can favour their import[138].

Nuclear membrane and ion channels

The nuclear envelope is a double lipid bilayer that is highly deformable. It behaves as fluid-like materials and rapidly reorganize in response to force, resulting in changes in membrane tension, curvature and surface area. These physical changes can influence lipid packing, bilayer thickness and conformations of membrane associated proteins[139]. Mechanosensitive ion channels such as piezo1 directly respond to these changes by altering their ion transport[140].

Nuclear actin

Nuclear actin has emerged as a multifaceted regulator of genome function through distinct structural and regulatory properties. Unlike well-organized filamentous networks observed in the cytoplasm, nuclear actin structures are short, highly dynamic and coupled to transcription, chromatin remodelling and DNA repair. It directly associates with all three RNA polymerases and is required for transcriptional initiation and elongation[141]. In the case of RNA polymerase II, it participates in the formation of pre-initiation complexes and in the recruitment of transcription factors[142]. It has also been shown to interact with chromatin remodelling complexes such as SWI/SNF and INO80, where it plays a role as a chaperone to mediate interactions between the complex and chromatin. Instead of having just scaffolding functions, actin appears to influence the enzymatic activity

of these complexes[143]. Nuclear actin has further been shown to influence chromatin mobility and their spatial organization into higher order structures as seen during gene transcription where actin polymerization events promote the repositioning of gene loci to transcriptionally permissive environments[141].

Regulation of actin dynamics is tightly controlled through its conformation state and nuclear import/export. Since actin lacks nuclear localization sequences (NLS), its binding to actin regulators such as cofilin which have conserved NLS sequences and importins are thought to be the primary mechanism driving its nuclear shuttling[144]. Other actin regulators such as formins further modulate their activity within and around the nucleus.

Formins at the nucleus

Many different formins have been found to act in and around the nucleus. We focused on FHOD1 and mDia2/DIAPH3.

FHOD1

FHOD1 (Formin homology domain containing protein 1) was initially characterized as a cytoplasmic actin nucleator and bundling factor, however, accumulating evidence has shown FHOD1 to also act around the nucleus by organizing perinuclear actin structures and interfacing with LINC. At the nuclear periphery, FHOD1 participates in the formation of transmembrane actin associated nuclear (TAN) lines which mechanically couple retrograde actin flow to the nucleus. It contributes to this primarily through its actin bundling activity by stabilizing dorsal actin cables that connect to nesprins, necessary for nuclear movement during cell migration[145].

FHOD1 has been shown to be activated by ROCK mediated phosphorylation, indicating it may also be sensitive to ECM mechanics. In addition, nuclear-cytoskeletal linkages involving FHOD1 were found to be necessary for cells to regulate their cytoskeletal tension in response to the stiffness of their environment, further suggesting mechanosensitivity of FHOD1[146].

mDia2/DIAPH3

Nuclear mDia2 (mammalian Diaphanous-related formin-2, also known as DIAPH3) has been shown to have important roles in transcriptional regulation by regulating transcription factor activity. In particular, mDia2 mediated actin polymerization promotes the activation of the serum response factor pathway (SRF) by releasing MRTF from G-actin, facilitating MRTF nuclear activity and SRF dependent transcription[147].

Beyond transcriptional control, mDia2 has been implicated in the DNA damage response. Nuclear actin polymerization is increasingly recognised as a critical

mediator of DNA repair, promoting the mobilization of damaged loci to repair permissive nuclear compartments[147].

mDia2 has also emerged as a regulator of nuclear architecture and stem cell fate. In MSCs, depletion of mDia2 results in a marked loss of lamin B1, altered nuclear mechanics and enhanced osteogenic differentiation. These observations highlight the importance of mDia2 dependent nuclear actin polymerisation in maintaining musculoskeletal integrity and cell fate decisions[148].

Collectively, these findings position mDia2 as a multifunctional nuclear formin that links actin dynamics to transcriptional regulation, genome maintenance and epigenetic stability.

Mechanical memory

Cells maintain tensional homeostasis through FAs and cytoskeletal organizations by balancing internally generated forces with external environmental stresses[149]. This dynamic equilibrium allows them to respond to changes in mechanical cues that arise during physiological processes such as wound healing through transient cellular responses, such as fibroblast activation to myofibroblasts, followed by a return to homeostasis through deactivation or apoptosis. However, prolonged exposure to certain mechanical cues can induce lasting changes in gene expression, cytoskeletal organization and signalling pathways even after the mechanical stimulus is removed, a phenomenon known as mechanical memory. In recent years, mechanical memory has been increasingly recognized as a key factor in the persistence of myofibroblasts in fibrosis and cancer[30].

Timescales of mechanical memory

Mechanical memory develops over several overlapping timescales, ranging from minutes to weeks. Immediate mechanotransduction through FAs and the cytoskeleton occurs within minutes where nascent adhesions can mature into FAs in 5-10min and disassemble within 10-20min or instead develop into fibrillar adhesions that are long lived[150-152]. Short actin filaments begin to associate with nascent adhesions and form elongated stress fibres associated with mature adhesions. These changes in FA composition and cytoskeletal tension activate signalling pathways discussed earlier such as RhoA and ROCK and the nuclear translocation of transcription factors[153, 154]. At these timescales, such cellular responses are transient and reversible, and the cell returns to its prior state upon removal of the mechanical stimulus.

In response to sustained mechanical stress, the internal architecture is continuously reorganised. Actin stress fibres thicken, nuclear shape changes and FAs mature. Transcription factors such as YAP/TAZ and MRTF-A modify gene expression profiles and epigenetic changes begin to take place[155, 156]. At this stage, cells may retain this primed state for a few hours or days after the stimulus is removed. At longer timescales, such as days to weeks, prolonged exposure to mechanical cues can lead to stable changes in gene expression that can be passed down through multiple rounds of cell divisions and affect differentiation and cell fate. Upon removal of the stimulus, cells may retain this phenotype for weeks or more[157, 158].

Apart from the duration of mechanical stress, magnitude of force is also a critical determinant of mechanical memory and its timescales. However, the relationship between stiffness and the acquisition of mechanical memory may not be linear as traction forces generated by cells on substrates of varying stiffnesses do not scale proportionally[159, 160]. Consistent with this, we also observed that persistent activation

did not increase linearly with stiffness. Instead, it peaked at 25kPa and decreased at higher stiffnesses.

Evidence of mechanical memory

Mechanical memory was first described in 2012 when Balestrini *et al.* observed that lung fibroblasts cultured on pathologically stiff substrates for 3 weeks maintained their myofibroblast state even after returning to healthy soft substrates. Similarly, long term culturing on soft substrates reduced myofibroblast activation when transferred to stiff substrates, showing that substrate priming has important implications in pathological and physiological processes[161].

Yang *et al*. observed that human mesenchymal stem cells (MSC) retained high irreversible YAP/TAZ activation along with the RUNX2 transcription factor on soft substrates following previous priming on stiff substrates, showing that stem cells possess mechanical memory[157]. Lee *et al*. found that MSCs that favoured neurogenic trans-differentiation on soft substrates displayed osteogenesis markers following prior exposure to stiff substrates[162]. Furthermore, MSCs cultured on micropatterned substrates that induced different cell geometries showed differential expression of osteogenic markers when transferred between stiffnesses, demonstrating that the architecture of the ECM also plays a role in mechanical memory driven lineage specification[163].

Meyer *et al*. showed that stem cells primed on soft substrates injected into rats with elbow injuries reduced anterior capsule fibrosis and improved their range of motion, highlighting a role for mechanical memory in stem cell therapies[164]. Tanaka *et al*. developed a substrate system that can synchronize MSC differentiation by inhibiting MSCs proliferation without losing lineage potential by frequently modulating substrate stiffness[165].

Hong *et al*. showed that fibroblasts subjected to mechanical stretch, as seen during skin grafts, can be activated in 3D tissues and develop mechanical memory based on how forces are transmitted through the ECM. By modulating the degree of tissue stretching, fibroblast activity and memory can be optimized to promote wound healing and reduce scarring[166].

Epithelial cells have also been shown to exhibit mechanical memory. Nasrollahi *et al*. reported that human mammary epithelial cells primed on stiff substrates migrated faster on soft substrates with large FAs, high actomyosin contractility and nuclear YAP[158]. Stiff matrices can even induce mechanical memory in poorly invasive oral squamous cell carcinoma cells to adapt mesenchymal phenotypes and become more migratory on soft substrates as shown by Moon *et al*[167].

Mechanisms underlying mechanical memory

How cells retain mechanical memory is not completely understood and is an area of active investigation. Emerging evidence suggests that cells can encode past mechanical experiences through a combination of processes such as persistent cytoskeletal remodelling, alterations in nuclear architecture and transcriptional changes that can be passed down through multiple generations. Such mechanosensitive transcriptional programs can lead to shifts in gene expression and epigenetic modifications while biochemical feedback loops can further stabilize these states, allowing cells to remember prior stresses even after the original stimulus is removed. Despite these advances, it remains unclear how these pathways integrate and whether a unifying mechanism of mechanical memory exists[159, 168].

Cytoskeletal and FA remodelling

ECM stiffness driven persistent reorganizations of the actin cytoskeleton have been reported where the formation of highly stable bundled actin stress fibres accompanied by actomyosin contractility persist for long periods of time. The incorporation of αSMA into stress fibres makes these highly ordered structures even more stable, creating architectural memory[159].

The meshcode theory is an emerging concept that proposes that cells store mechanical memory directly within molecular conformations and architectures of adhesion proteins and their higher order structures. Mechanosensors that undergo force dependent conformational changes such as talin are engraved with the pattern of their folding and unfolding states, retaining a history of their mechanical loading. Protein stretching and binding reinforces the adhesion, where force induced structural rearrangements create a stable network or "mesh" that encodes prior mechanical conditions as physical patterns or "codes" at the adhesion site. Further, mature FAs disassemble more slowly and maintain stronger cytoskeletal coupling. They can leave behind a predisposed architecture that facilitates rapid reassembly. As a result, cells previously exposed to stiff environments may be able to exhibit enhanced FA formation and generate higher traction forces[160].

Transcriptional feedback loops

Transcriptional coactivators YAP/TAZ function downstream of the Hippo signalling pathway and are strongly regulated by physical factors such as ECM stiffness, ligand density, cell shape and cytoskeletal tension[169]. When cells experience stiff or highly stretched environments, YAP/TAZ translocate to the nucleus in response to stress fibre formation. Prolonged exposure to stiff environments can lead to their sustained nuclear retention and the development of gene expression patterns that persist even after the removal of the inducing mechanical stimuli. Further, the nuclear retention of YAP/TAZ can be sustained by positive feedback loops where YAP/TAZ target genes reinforce cytoskeletal tension, thereby stabilizing their own activity[158, 170].

MRTF-A is another transcription factor that contributes to mechanical memory by reinforcing and stabilizing cellular responses based on prior mechanical stimulus. It localizes to the nucleus upon release during actin polymerization on stiff ECMs and drives the expression of cytoskeletal and contractility related genes. Upregulation of αSMA for example increases cellular contractility which maintains actin polymerization and in turn keeps MRTF-A active in the nucleus[159].

Finally, slow nucleo-cytoplasmic transport of transcriptional regulators occurring over several days can also influence mechanical memory where a change in mechanical stimulus does not induce a sudden change in localization of transcription factors[171].

Nuclear mechanotransduction

Force induced adaptations of nuclear components such as the nuclear lamina have been shown to persist for several days after mechanical stimulation. These long-lived alterations modify nuclear mechanics and gene regulation, directly contributing to the storage of mechanical memory[172]. Similarly, recovery from force induced chromatin condensation depends on the duration of external stress. Heo *et al*. showed that loads acting on the nucleus for 3h resulted in condensed chromatin, while cells exposed to the load for less than an hour could reverse their condensation states[173].

Epigenetic modifications

Force induced changes in nuclear structures and transcription factor activity result in persistent epigenetic modifications that have been shown to drive mechanical memory through histone modifications, DNA methylation and non-coding RNAs that ultimately regulate chromatin accessibility, organization and condensation.

Mechanotransduction pathways have been shown to interact with **DNA methyltransferases** that catalyse the establishment of new methylation marks on CpG islands near gene promoters. Methylation at these regions leads to gene silencing while if unmethylated, active gene transcription can take place[174]. In CAFs, atypical DNA methylation profiles have been observed in a number of cancers. Downregulation of tumour suppressors like TGFBR2 in prostate cancer and PTEN in breast cancer have been reported[175, 176]. Pancreatic CAFs displayed high methylation patterns at cytokine signalling suppressor genes[177]. Although methylation patterns are reversible, the level of reversibility progressively declines with the duration of mechanical stress[159].

MicroRNAs (miRNA) contribute to mechanical memory by targeting and silencing mRNAs. They are highly stable and can persist for several days. miR-21 and miR-29 are well studied miRNAs that are upregulated in stiff environments and target inhibitors of fibrosis. In addition to repressing regulators, these miRNAs can sustain activation of pro-contractile and pro-fibrotic pathways by regulating the

MAPK/ERK pathway[178, 179]. YAP/TAZ and MRTF-A have also been shown to regulate miRNA transcription. In turn, the miRNAs can target components of these pathways, forming reciprocal regulatory circuits that persist for extended periods of time[180].

Chromatin modifications mediated by enzymes such as **histone acetyltransferases (HATs)**, which induce chromatin decondensation, and **histone deacetylases (HDACs)**, which induce chromatin condensation, are mechanosensitive (Figure 13). Chromatin remodelling in response to stiff environments in MSCs has been shown to be mediated by a decrease in HDACs and an increase in HATs, determining MSC cell fate[172, 181]. Consistent with this, Killaars *et al*. showed that mRNA expression levels of HDAC1, HDAC2, HDAC3 and HAT1 differ significantly between MSCs that acquired substrate stiffness memory and those that did not, suggesting that chromatin accessibility plays a central role in regulating cell fate[181].

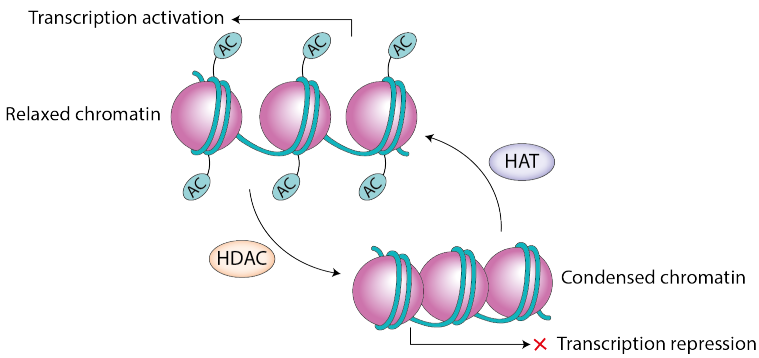


Figure 13: Chromatin accessibility states are regulated by enzymes.
Chromatin modification enzymes, HATs and HDACs, induce chromatin opening and condensation respectively. Histone acetylation by HATs reduces the positive charge of histone tails and inhibits their binding to negatively charged DNA, making DNA more accessible to transcription factors. Image created with Adobe Illustrator.

In the context of fibrosis and myofibroblast activation, it has been suggested that HATs enhance chromatin accessibility, promoting pro-fibrotic gene expression and fibroblast activation, whereas HDACs reduce chromatin accessibility and contribute to the silencing of anti-fibrotic genes. The inhibition of class I and II HDACs in fibroblasts have been shown to reverse stiffness mediated persistent myofibroblast activation, with cells reverting to less activated states upon return to soft environments. Similarly, HDAC inhibition in CAFs suppresses their activation and dampens the response of fibrotic fibroblasts and CAFs to TGF-β and TNFα[182, 183]. Furthermore, Walker *et al*. demonstrated that increasing chromatin condensation enabled transiently activated fibroblasts to acquire persistence more rapidly. These

findings suggest that chromatin accessibility also plays an important role in regulating cell phenotype switching.

HDACs and HATs have been shown to be regulated by FAs and mechanosensors. Phosphorylation of class II HDACs like HDAC5 by FAK affects their nuclear translocation[184]. Conversely, HDACs can affect FA dynamics. Inhibition of HDAC1 and HDAC2 reduced the expression of talin-1 and consequently the activity of β1 integrins[185]. Just as mechanical stimuli can be transmitted to the nucleus through the cytoskeleton to regulate HAT and HDAC activity, HATs and HDACs can in turn regulate the cytoskeleton and affect signal transmission. Microtubules in particular are regulated by HAT1 and HDAC1 activity. HDAC3 has been shown to regulate ROCK activity and SAHA, a HDAC inhibitor, had inhibitory effects on the MAPK/ERK pathway, suggesting that mechanotransduction pathways may also have acetylation as an important regulatory posttranslational modification[186].

Collectively, these studies highlight the close relationship between mechanotransduction, epigenetic regulation and nuclear architecture. Notably HDACs and lamin have both been implicated in modulating chromatin accessibility by regulating its open and closed states, suggesting that chromatin state may be an important factor by which mechanical signals may be translated into long lasting changes in gene expression.

Knowledge gaps

Although significant progress has been made in understanding FA mediated ECM sensing, fundamental questions about how ECM properties such as ECM stiffness, ligand density and spatial organisation are encoded within FAs to generate specific cellular responses remain unanswered. Previous studies have identified extensive repertoires of FA components, mapped their nanoscale localizations and uncovered various mechanosensing mechanisms such as catch bonds and force induced conformational changes. Yet these discoveries do not fully explain how cells discriminate between distinct ECM properties with such sensitivity and specificity. In particular, it remains unclear whether features beyond protein composition and localisation, such as the orientational order and alignment of FA components, actively contribute to mechanosensing and how these structural properties encode information about the physical environment.

Another unresolved question is how this encoded information gives rise to heterogenous mechanosensing responses across spatially distinct FA populations. Although FAs are recognised as heterogenous populations, the mechanisms that establish and maintain functionally distinct FA subpopulations remain poorly understood. In particular, the role of non actin cytoskeletal regulators, such as septins, in organising FA structures and modulating their sensitivities to different ECM cues is unknown. Whether septins generate specialised FA populations with distinct mechanosensing properties represents an important gap in understanding how cells decode complex physical environments.

Finally, it remains unclear how mechanical stimulation sensed at FAs is converted into stable, self-sustaining changes in cell state. While FAs and downstream mechanotransduction pathways initiate force signalling, the molecular mechanisms that transmit physical information from integrins to the nucleus and remodel chromatin remain incompletely understood.

Together these gaps span multiple scales, from nanoscale molecular organisation within individual FAs to the long-term epigenetic reprogramming of the nucleus. This thesis addresses them in an integrated manner, investigating how FA architecture encodes ECM information, how non-actin regulators shape FA heterogeneity and mechanosensing specificity, and how sustained mechanical signals are transmitted to the nucleus to establish persistent fibroblast activation through chromatin remodelling.

Project aims

The aims of the papers included in this thesis were:

Paper I: To determine how the nanoscale alignment of integrins and F-actin in focal adhesions contribute to cellular mechanosensing of the ECM.

Paper II: To investigate how the cytoskeletal protein septin 7 is regulated by ECM mechanics within focal adhesions and to determine its role in cellular mechanosensing and functional responses in fibroblasts.

Paper III: To elucidate the molecular mechanisms by which ECM stiffness induces persistent activation in fibroblasts. The study sought to determine how mechanical cues are transmitted through β1 integrins and mDia2 formin dependent pathways to remodel nuclear architecture and chromatin organization to establish stable activated fibroblast phenotypes.

Methods

Immunofluorescent staining

Immunofluorescent staining is a technique used to visualize cells, tissues and their components. Typically, a primary antibody binds to specific antigens followed a fluorescently labelled secondary antibody that recognizes and binds to the primary antibody, enabling their visualization with fluorescence microscopy. General steps involved fixation with paraformaldehyde (PFA) to preserve cellular structures, permeabilization with triton-x to allow antibodies to enter the cell, aldehyde quenching with glycine to neutralize reactive groups and reduce background signal, a blocking step with BSA to reduce nonspecific antibody binding and finally incubation with primary and secondary antibodies. Samples stained on gels were mounted using a sandwich technique where a cover glass was placed on top and imaged upside down to keep the sample within the working distance of objectives.

Two buffers were used based on the protein target. Phosphate buffered saline (PBS) is an isotonic buffer that helps preserve general cell morphology and protein structures during staining. However, PBS does not stabilize the cytoskeleton and actin filaments can depolymerize. Cytoskeletal buffer, which is formulated to preserve actin structures, was used in experiments investigating FAs, actin anisotropy and αSMA stress fibres. It contains EGTA which chelates calcium ions that can induce actin depolymerization and Mg^{2+} that stabilizes ADP actin and prevent its depolymerization from filaments.

Hydrogel preparation

Homemade hydrogels of different stiffness were used to perturb cell spreading and FA growth in paper I and II. Standard tissue culture plates are far stiffer than most biological tissues and as a result, cells can adopt non-physiological phenotypes. Gels of defined stiffness provide more physiologically relevant environments.

We adopted a polyacrylamide-based hydrogel system modified from a previously described method[187]. Gel stiffness was tuned by varying the ratio of bis-acrylamide, a crosslinking agent and acrylamide. Hydrogels were formed through free radical

polymerization initiated by ammonium persulfate, which generates sulfate radicals that trigger acrylamide chain formation and crosslinking with bis-acrylamide. Because APS decomposition is slow on its own, Tetramethylethylenediamine (TEMED) was added as a catalyst to accelerate radical generation and create a 3D gel. To ensure uniform polymerization, gel solutions were degassed to remove dissolved oxygen which can inhibit polymerization by scavenging free radicals.

For imaging applications, the hydrogels were covalently attached to cover glasses. Prior to polymerization, coverslips were treated with glutaraldehyde to coat them with reactive surface groups capable of forming covalent bonds with polymerizing gels.

The surface of the gels was functionalized to enable the binding of ECM proteins such as fibronectin. This was done through photoinitiated modifications of the gel surface. Lithium phenyl-2 4 6-trimethylbenzoylphosphinate (LAP) was used as a photo initiator that generates free radicals and allow spatial patterning when exposed to UV light. N-hydroxysuccinimide (NHS) chemistry was used to convert carboxyl groups into NHS esters that can interact with amines on ECM proteins to form stable amide bonds. The addition of bis-acrylamide in this step can help the covalent trapping of these functional molecules at the gel surface.

Surface functionalization is one of the most fragile steps in the workflow with high failure rates. A major source of failure is the instability of NHS esters that undergo rapid hydrolysis in solutions and the scavenging of free radicals produced by LAP by oxygen. Although degassing is used to mitigate this, oxygen can renter during handling. Poly(ethylene glycol) (PEG) based hydrogels that already have NHS are easier to functionalize. Collagen hydrogels that provide cell adhesion sites and physiologically relevant are limited in stiffness control and expensive. Due to these drawbacks, we switched to using commercially produced polyacrylamide hydrogels in paper III.

Microscopy

Microscopy plays an important role in cell biology because it provides spatial and structural information that cannot be obtained from biochemical assays. Protein distribution, their location and their organization into higher order structures often determines cellular function and are directly observable with microscopy. Microscopy supports morphological analysis such as changes in cell shape, spreading, polarity and cytoskeletal architecture that are central to many biological processes and show cell to cell heterogeneity. It also provides correlative approaches, where structural, functional and biochemical information can be extracted together.

Widefield epifluorescence microscopy

In Widefield epifluorescence microscopy, the same objective lens is used for excitation and detection. Excitation light from a LED or laser source is directed through an excitation filter and reflected by a dichroic mirror into the objective. The objective focuses the light onto the specimen and excites fluorophores. Emitted fluorescence is collected through the same objective, transmitted through the dichroic mirror and detected by a camera. Since the entire field of view is illuminated and detected, the image contains in focus and out of focus fluorescence from all planes within the thickness of the specimen.

Since Widefield systems collect all emitted photons, they provide higher signal to noise ratios when using dim fluorophores. They are suitable for high speed and high throughput imaging with low phototoxicity. The primary limitation is the presence of out of focus light in thick specimens due to emission of photons form regions above and below the focal plane that are collected with similar efficiency as in focus emission. This reduces the axial resolution. However, in thin samples, out of focus contributions are minimal.

Widefield epifluorescence microscopy is well suited for morphological phenotyping such as cell and nuclear area measurements, stress fibre analysis and nuclear lamina morphological quantifications in flat adherent cells. The specimen thickness is in the order of a few micrometers and the axial range of the point spread function (PSF) is less problematic as most of the fluorescence originates from a narrow z range. Further, high signal to noise ratios from whole cell fluorophore emissions helps visualization and segmentation during image analysis. This was helpful when quantifying αSMA as its expression is extremely heterogenous with some cells expressing very low amounts.

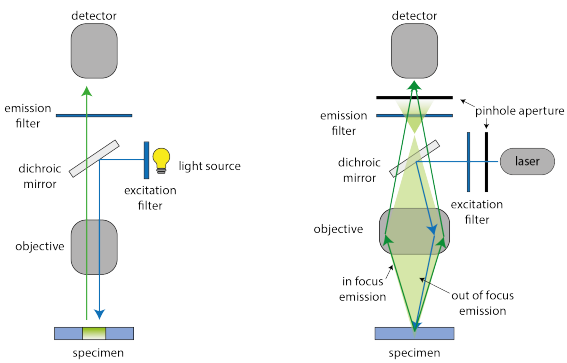


Figure 14: Image generation in widefield epifluorescence and confocal microscopy.
Widefield microscopy illuminates the entire sample and collects light from all planes within the sample (left). Confocal microscopy illuminates a diffraction limited spot within the sample and reduces the collection of out of focus light by directing the emission through a pinhole (right). Image created with Adobe Illustrator, adapted from [188].

Point scanning confocal microscopy

In point scanning confocal microscopy (PSCM), excitation light is focused to a diffraction limited spot within the specimen and raster scanned across the field of view. Fluorescence emission is directed through a pinhole at the image plane where the object is in focus before reaching the detector. The pinhole acts as a filter, rejecting photons from out of focus planes.

The confocal PSF is much narrower in the axial dimension than widefield systems, enabling optical sectioning for volumetric reconstructions. This is important for thick samples and dense intracellular environments such as chromatin in the nucleus. The narrow PSF can resolve peripheral chromatin enrichment from signal deeper within the nucleus. The reduced out of focus background fluorescence from other nuclear planes improves contrast and are suitable for colocalization studies.

PSCM is limited by the diffraction barrier (200-250nm laterally and 500-800nm axially). Chromatin and histone modification clusters are at scales below the confocal limit and produce mildly blurred averages of structurally distinct features. Better alternatives are super-resolution approaches such as STED microscopy that reduce the size of fluorescence excitation volumes. A depletion bean suppresses fluorescence emission from around the excitation spot, shrinking the PSF. STORM microscopy achieves even higher spatial resolution by separating the emission of individual fluorophores and reconstructing their positions. However, both techniques are experimentally more demanding and can have large acquisition times, risking photobleaching and less suited to high throughput screening.

Polarization microscopy – Emission anisotropy TIRF

Emission anisotropy-Total Internal Reflection Fluorescence (EA-TIRF) microscopy was used to study the molecular ordering of integrins and actin within FAs in paper I. EA-TIRF is a specialized technique that uses the polarization state of emitted fluorescence to extract information about molecular orientation. In standard TIRF microscopy, light undergoes total internal reflection at the interface between a high refractive index medium such as glass and a lower one such as water. This creates an evanescent field of 100-200nm at the surface. Only fluorophores within this region are excited, thereby reducing background fluorescence (Figure 15). This makes TIRF microscopy ideal for studying membrane proteins such as FAs.

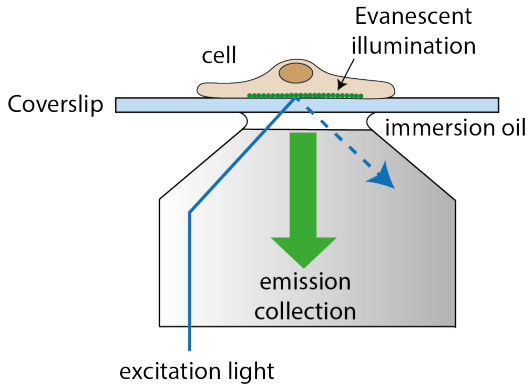


Figure 15: TIRF schematic.
The excitation light undergoes total internal reflection at the interface between a high refractive index medium and a low one, creating a narrow evanescent field within which fluorophores are excited. Image created with Adobe Illustrator, adapted from [189].

Since fluorescence emission is not isotropic, the polarization of emitted photons carries information about how molecules are oriented and how freely they rotate. This is because a fluorophore does not absorb light equally from all directions. Its excitation is governed by a transition dipole moment, a vector that defines the direction along which the molecule most efficiently interacts with the electric field of incoming light. The probability that a fluorophore molecule absorbs a photon of light and emit fluorescence depends on how its transition dipole aligns with the electric field of linearly polarized excitation. Quantitatively, the excitation probability follows a $\cos^2\theta$ dependence where θ is the angle between the dipole moment and the electric field vector. Therefore, if a fluorophore is oriented parallel to the excitation polarization ($\theta = 0°$), the excitation is most efficient, and they absorb more photons and emit stronger fluorescence in the same direction. Conversely, if a fluorophore is oriented perpendicular to the excitation polarization ($\theta = 90°$), the excitation is minimal, and they emit very little fluorescence perpendicular to the excitation.

Fluorescence anisotropy is defined as:

$$r = \frac{I_{\parallel} - G.I_{\perp}}{I_{\parallel} + 2.G.I_{\perp}}$$

Where $I_{\parallel}$ and $I_{\perp}$ are the emission intensities detected parallel and perpendicular to the polarization axis (excitation polarization). These components are measured by splitting the emitted light into two orthogonally polarized detection channels (setup shown below) using a polarizing bean splitter (Figure 16). G is the g-factor

correction to account for unequal detector sensitivities. The total intensity (denominator) must account for all 3 mutually orthogonal polarization directions: one parallel and two equivalent perpendicular directions to the excitation polarization (x, y and z axis). Since only one of them is detected, $I_{\perp}$ is multiplied by 2 to obtain total perpendicular emission.

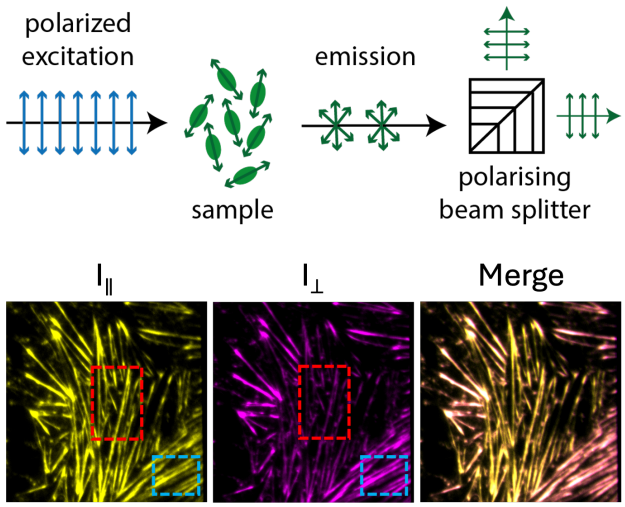


Figure 16: Schematics of EA-TIRF (top) and EA-TIRF images (bottom).
Depending on the orientation of a fluorophores transition dipole w.r.t the axis of polarization excitation, fluorophores will absorb and emit differently. When a Fluorophore's dipole is aligned parallel to the excitation polarization, it absorbs light most efficiently and more likely to emit strongly in the parallel polarization than in the perpendicular one (red box). Fluorophores with dipoles oriented perpendicular to the excitation polarization are less efficiently excited and emit light polarized parallel to its own dipole axis (blue box). Because excitation preferentially selects fluorophores aligned with the excitation field, the overall detected fluorescence remains biased towards the excitation polarization ($I_{\parallel}$).

To describe how the mean anisotropy value varies with angle (θ), the following equation was used:

$$r = A.\cos^2(\theta + p) + C$$

Where A is the amplitude that reflects the degree of order and determines how strongly anisotropy varies with angle. Large A indicates well aligned molecules while a small A indicates randomly oriented molecules. θ is the angle between the excitation polarization and FA long axis. p is the phase offset that accounts for the fact that maximum signal doesn't necessarily occur at θ = 0°. It reflects the average orientation of the dipoles. So, (θ + p) shifts the cos2 function to reflect reality. C is the baseline anisotropy from any isotropic background signal[97].

Since anisotropy measurements depend on fluorophore behaviour, the fluorophores rotational freedom must be restricted relative to the timescale of fluorescence emission. Therefore, integrin constructs expressing constrained GFP proteins are used in our studies. The cells must also be plated sparsely as crowding can result in environmentally induced molecular ordering. EA-TIRF is further restricted to substrates with refractive indices similar to glass and cannot be used with commonly available polyacrylamide hydrogels that mimic physiological stiffness. Alternative approaches to overcome this include 'time resolved fluorescence lifetime imaging microscopy' that probes fluorescence lifetimes instead of just intensity ratios. 'Highly inclined and laminated optical sheet microscopy' works with PA gels and only penetrates a few microns into the sample, enabling surface imaging like TIRF. It can be combined with polarization splitting for anisotropy measurements.

Transfection

Cell transfections enable the delivery of exogenous DNA/RNA into cells to modify gene and protein expression levels. Transient transfection systems can broadly be categorized into chemical and physical methods. Chemical methods such as lipofectamine were used to overexpress the FA protein mCherry-paxillin and YFP-septin7 and to knockdown FHOD1 and SMIFH2 formins and septin7. Physical methods such as the Neon electroporation system was used to overexpress constrained-GFP α_v integrins.

Lipofectamine based transfections are driven by synthetic lipids that that package nucleic acids into complexes that can transverse the cell membrane and release them into the cytoplasm. In the cytoplasm, they evade endosomal degradation through lipid reactions and siRNA complexes are incorporated into 'RNA-induced silencing complex' that results in translation repression. For plasmid DNA, an additional step of entry into the nucleus is required which depends on cellular processes. Nuclear envelope breakdown during mitosis enables plasmid translocation while intracellular trafficking mechanisms and active transport are employed during interphase. The Neon electroporation system introduces plasmids into cells by applying electric pulses that temporarily disrupts the plasma membrane, allowing plasmids to enter the cytoplasm. This was useful for plasmids that were difficult to transfect such as the integrin plasmids that only worked with electroporation.

Image analyses

Image analysis enables the extraction of quantitative information from visual data. It can also be used to overcome limitations of imaging systems such as noise, uneven illumination and diffraction. For example, deconvolution algorithms can improve contrast and resolution by reducing blurring from out of focus signal and denoising algorithms enhance signal detection in low fluorescence conditions.

Image analysis platforms such as FIJI and Cell Profiler were used to quantify YAP N/C ratios, cell and nuclear morphological quantifications. They are free to use and allow the construction of analysis pipelines without requiring extensive programming knowledge. Pipelines written in Julia and Python were used for more complex analysis involving lamin, histone and αSMA images, where customized approaches were built to analyse these datasets.

Paper summaries

Paper I

Extracellular matrix sensing via modulation of orientational order of integrins and F-actin in focal adhesions

The ability of cells to sense and respond to the ECM governs their function and behaviors. A key mechanism underlying ECM sensing is mechanotransduction, the process by which cells convert mechanical cues into biochemical signals. Central to mechanotransduction are integrin-based FAs that link the ECM to the cells cytoskeleton and enable cells probe their environment. Although extensive research has established the importance of FA signalling and mechnanosensor activity, little is known about the features that determine their organization and how their alignment contributes to ECM sensing. In this paper, we find that α_v integrins and F-actin undergo ECM density mediated changes in orientational order independent of myosin-II activity. A molecular-clutch model demonstrated that ECM ligand sensitivity increases the most by increasing the probabilities of both integrin activation and directional catch bonds and ECM density sensing independent of ECM stiffness.

Results

Orientational order of integrins and F-actin varies with ECM density and integrin activation

We first tested if the orientational order of α_v integrins and F-actin was sensitive to changes in ECM density and found that MEFs plated on glass dishes coated with 0.1, 1 and 10 ug/ml of fibronectin (FN) showed increasing α_v integrin and F-actin orientational ordering with FN density. The orientational ordering of F-actin was 5 times more than integrins at the same FN density (Figure 17). These changes corresponded with changes in cell area and YAP N/C ratios.

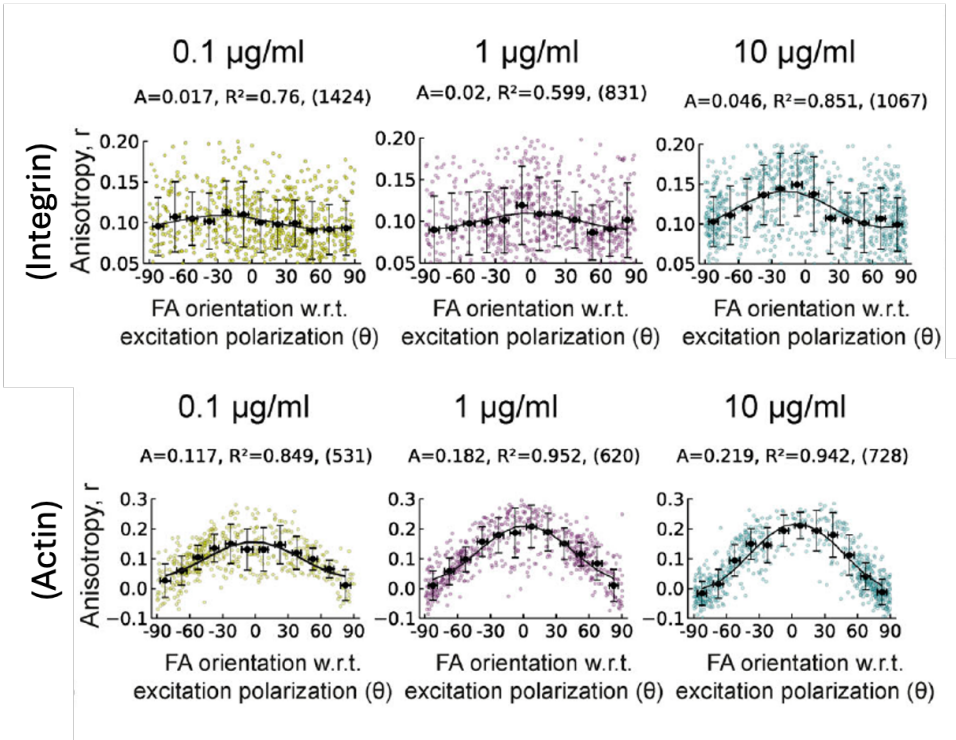


Figure 17: αv integrin and F-actin orientational order changes with ECM density
Mean integrin and F-actin anisotropy (r) in FAs versus FA orientation fit to the cos2 function r = C + A·cos2(γ + θd) for cells plated on each FN condition. "A" is the amplitude of the curve, "R2" is the R2 value of the fit, and the number of FAs is given in parenthesis. Error bars represent SD.

We then confirmed if the orientational order of F-actin depended on integrin activation by plating cells on low FN densities in the presence of Mn2+, an integrin activator. We found that cells under this condition had bigger FAs with F-actin displaying a twofold increase in orientational order than nontreated cells. These results indicate that ECM density modulates the orientational order of α_v integrins and F-actin via integrin activation.

Two mechanisms of modulating orientational order: Myosin II dependent and independent

We next investigated the role of myosin II-mediated contractility in FA mediated ECM sensing. Since nascent adhesions are couped to myosin II independent actin polymerization and focal adhesions are couped to myosin II dependent actin contractility, we binned the FAs from the previous experiment into small nascent adhesions, intermediate adhesions and large focal adhesions based on their size. We found that small adhesions had highly disordered F-actin on low FN densities but

increased greatly on high FN densities (~2.7 fold change) whereas, large adhesions had ordered F-actin on low FN densities which increased mildly on high FN densities (~1.45 fold change). These results indicate that nascent adhesions are the most sensitive to change in ECM ligand density.

We then tested if myosin II was required for ECM sensing. Cells treated with myosin II-inhibitor blebbistatin washouts displayed small FAs with low F-actin orientational order at all FN densities compared to untreated cells. Interestingly, loss of myosin II did not completely abrogate FN density-dependent changes in orientational order of F-actin as there was ~1.18 fold increase in F-actin orientational order between low and high FN densities. These results suggest that myosin II is required for the enhanced orientational order of F-actin in all FAs resulting in increased sensitivity of cells to ECM densities.

Molecular clutch model captures specificity between sensing of ECM density vs ECM stiffness

We next explored possible mechanisms that could drive the changes in orientational order of F-actin to enable sensitivity to ECM density independent of myosin II activity. A computational model of nascent adhesion assembly was extended to include ECM density-dependent activation of integrins and actin orientation-dependent kinetics of vinculin. The resulting number and lifetime of clutches in the different conditions that were tested experimentally were evaluated and showed that clutches were ligated for a longer time when orientational ordering of both FA components (integrin and F-actin) was maximized. Increasing either parameter in isolation narrows the range of the fraction of clutches, resulting in less sensitivity to ECM density.

Because myosin II contractility is coupled to ECM stiffness, we hypothesized that FN density sensing was distinct and independent of ECM stiffness. Three stiffness with three FN densities were tested with the model which showed that at different stiffnesses, sensitivity to ECM density also increased the most when orientational ordering of both integrins and F-actin were increased simultaneously. The model showed a biphasic response across different stiffnesses for the same ligand density. This occurred because when substrate forces were too high, adhesions disassembled instead of stabilizing.

Overall, the orientational order-based motor clutch model predicted ECM density sensing independent of ECM stiffness with a biphasic response across the stiffness range for a given ECM density. Experimental quantification of the cell spread area and YAP N/C ratio across all these conditions showed a good match with the prediction from the model, indicating that ECM-dependent changes in the orientational order of FA components and regulation of directional catch bonds

provide a robust mechanism for fine-tuning the sensitivity of cells to ECM density, independent of ECM rigidity.

Conclusions

The main conclusions of paper I are:

ECM Density Alters Molecular Alignment: Both α_v integrins and F-actin undergo changes in orientational order in response to ECM ligand density, with these changes depending on integrin activation.

Nascent Adhesions Are Highly Sensitive: F-actin orientational order in nascent adhesions is particularly sensitive to changes in ECM ligand density, indicating that early adhesions are the most responsive to ECM density.

Dual Contractility Regimes: Orientational order is established through two distinct mechanisms: an actin polymerization-dependent, myosin-II independent pathway at low ECM densities, and a myosin-II-dependent pathway that enhances cellular sensitivity at higher ECM densities.

Decoupling of Matrix Density and Stiffness Sensing: A molecular-clutch model suggests that ECM density and substrate stiffness sensing are sensed through distinct mechanisms. ECM ligand density is sensed primarily through nanoscale reorganization of FA components whereas substrate stiffness is detected predominantly through force-dependent mechanisms involving myosin.

Paper II

ECM-dependent regulation of septin 7 in focal adhesions promotes mechanosensing and functional response in fibroblasts

In the past few years, FA proteomics has revealed over 2000 proteins to be associated with the adhesome, with a large portion of these having unknown functions at FAs. While several mechanisms of FA mediated ECM sensing have been identified, mechanisms that fine-tune the sensitivity and specificity of ECM sensing and cellular response is still not completely known and may be mediated by one these functionally elusive FA proteins. To address this, we focused on investigating the role of Sept-7 which is one of the most enriched septins identified in the adhesome. By using TIRF microscopy, we found that Sept-7 forms distinct structures on the ventral surface of cells that localize to and in proximity of FAs. In addition to location specific architecture, Sept-7 localization and function was mediated by integrin activation and spatially and temporally distinct within FA subpopulations. Through these mechanisms, Sept-7 promotes the sensitivity of fibroblasts to regulate the physical properties of the ECM.

Results

Septin 7 has distinct FA localizations regulated by integrin activation

We first investigated the localization of septin 7 at FAs in MEFs by TIRF microscopy and found that at perinuclear FAs below the nucleus, septin 7 localized around stress fibres near paxillin rich FAs and colocalized as punctate structures with paxillin at peripheral FAs near the front of the cell. Line scans across the FAs confirmed this observation. The average intensity peak of septin 7 was behind paxillin in perinuclear FAs while the peak was closer to paxillin in peripheral FAs.

To explore the link between septin 7 recruitment and ECM signalling, MEFs were plated on different concentrations of fibronectin and PLL to modulate integrin activation. Cells on PLL, which prevents integrin activation, showed diffused FAs and septin 7 signal. Cells on 0.1 μg/mL FN displayed septin 7 bundles at perinuclear FAs as seen on 10 μg/mL FN controls but diffused septin 7 punctas at peripheral FAs. Increasing integrin activation with MnCl2 increased the area of septin 7 structures at peripheral FAs. Together, these results show that septin 7 has distinct localizations at perinuclear and peripheral FAs and is regulated by integrin activation.

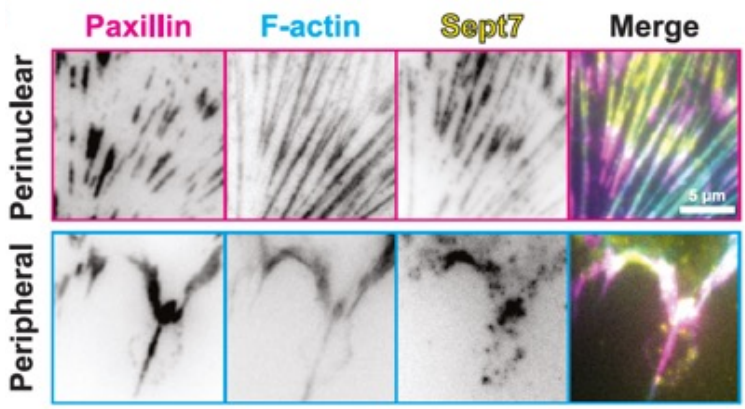


Figure 18: Sept-7 localizes in spatially distinct patterns in FA sub-populations
At perinuclear FAs, septin 7 localized around stress fibres near paxillin rich FAs and colocalized as punctate structures at peripheral FAs.

Septin 7 enhances fibroblast sensitivity to changes in ECM cues

Since septin 7 is strongly associated with FAs and ECM signalling, we next investigated its role in FA maturation and ECM sensing. Septin 7 KD resulted in smaller peripheral FAs and a loss of perinuclear FAs. Loss of ventral stress fibres associated with perinuclear FAs below the nucleus was also observed. Live imaging with mCherry-paxillin showed that septin 7 KD greatly reduced the lifetimes of perinuclear FAs but had no effect on peripheral FA lifetimes, indicating that septin 7 promotes the stabilization of perinuclear FAs by increasing their lifetimes.

We next investigated the role of septin 7 in ECM density and stiffness sensing. MEFs plated on soft 0.4kPa and stiff 60kPa hydrogels only had perinuclear septin bundles on 60kPa hydrogels. Septin KD cells on soft hydrogels showed similar cell spread areas as NT controls on soft hydrogels while septin 7 KD cells spread to lesser extents on stiff hydrogels. Similarly, septin 7 KD cells plated on low fibronectin concentrations (0.1 ug/ml) had similar cell spread areas as NT controls but displayed reduced similar cell spread areas on high fibronectin densities (10 ug/ml) with low stress fibre formation. These results suggest a role for septin 7 at perinuclear FAs for durosensing and at peripheral for haptosensing.

Septin 7 contributes to ECM remodelling

Since fibrillar FAs at the perinuclear region are known to be involved in ECM remodelling, we next tested if septin 7 at perinuclear FAs was important to this process. Septin 7 KD plated on fibronectin coated plates showed reduced ECM clearance when compared to NT control cells. This suggests an additional role for septin 7 in mediating ECM remodelling, possibly through their regulation of perinuclear FAs.

Conclusions

The main conclusions of paper II are:

Subpopulation Localization: Septin 7 localizes to spatially and temporally distinct regions within FA subpopulations. In peripheral FAs, septin 7 structures are elongated and within the core of the FA. In perinuclear FAs, septin 7 forms bundles stabilized at the rear of the FA.

ECM and Integrin Dependence: septin 7 recruitment and structural arrangement of are regulated by ECM binding and integrin activation. Low ECM densities can induce perinuclear bundles, whereas high ECM density and high integrin activation are required for peripheral septin 7 elongation.

FA Regulation: Septin 7 depeltion reduces the number, size, and lifetime of perinuclear FAs, indicating that septin 7 is crucial for their maturation and stability. It also minorly impacts the elongation of peripheral FAs.

Functional Mechanosensing & Remodelling: Septin 7 enhances fibroblast sensitivity to physical cues from the ECM. Septin 7 depletion impairs the ability of cells to increase their cell spread area in response to increased ECM stiffness and density and significantly reduces their capacity to remodel the ECM.

Paper III

ECM-Stiffness Mediated Persistent Fibroblast Activation Requires Integrin and Formin Dependent Chromatin Remodelling

With the recent emergence of mechanical memory as an important factor in fibrosis progression, we aimed to identify the mechanisms that drive mechanical memory in CAFs. Using a hydrogel-based system, we defined stiffness conditions that regulate CAF activity. CAFs on stiff 25kPa hydrogels exhibited an activated phenotype characterized by the presence of αSMA stress fibres, whereas those on soft 0.5kPa hydrogels remained inactive and lacked these structures. CAFs exposed to stiff substrates for prolonged periods of time retained αSMA stress fibre expression even after transfer to soft substrates, indicating the acquisition of mechanical memory and persistent activation. We find that this sustained activation is driven by two mechanotransduction pathways, one through integrins and other through formins, which converge to regulate lamin-chromatin interactions. Blocking either of these pathways inhibits persistent activation but this can be rescued by the inhibition of histone deacetylases, indicating a role for epigenetic mechanisms in mechanical memory.

Results

Stiffness driven persistent activation requires extended culturing and chromatin repositioning

We first sought to establish the time required to for CAFs to become persistently activated by culturing them for on stiff (25kPa) hydrogels for different periods of time. Short term exposure to stiff hydrogels induced typical activation markers, but this state was reversible when cells were transferred to soft (0.5kPA) hydrogels. In contrast, extended culture on stiff substrates led to a stable activated phenotype that persisted even after transferring to soft substrates. This persistence not only requires mechanical signals but also stiffness dependent secreted factors, indicating that both biochemical signalling and mechanical cues are important for persistent activation.

We then investigated changes in chromatin remodelling during persistent activation. Since ECM stiffness has previously been shown to affect chromatin condensation states, we perturbed chromatin acetylation with HDAC and HAT inhibitors and found that while inhibiting chromatin acetylation did not affect transient activation, inhibiting HDACs reduced persistent activation, suggesting a role for HDACs in the acquisition of persistent fibroblast activation.

Persistent activation requires integrin activation and mDia2 activity

We next investigated the role of ECM receptors integrins in persistent activation and found that sustained engagement and activation of β1 integrins during prolonged exposure to stiff substrates is required as blocking β1 integrins inhibited persistent activation. This was observed along with altered remodelling of the nuclear lamina and redistribution of chromatin at the nuclear lamina during prolonged culturing.

Further, we found that mDia2 activity during priming on stiff substrates is also required for persistent activation. Unlike integrins, mDia2 does not act by altering the nuclear lamina. Instead, it appears to function by organizing actin which in turn influences chromatin organization.

HAT inhibitor rescues integrin and mDia2 blocking

Since inhibiting either β1 integrins or mDia2 activity inhibits persistent activation and both converge on altering chromatin organization at the lamina, we tried to rescue these effects by altering chromatin acetylation states. HDAC inhibition rescued the effects of integrin and mDia2 blocking, however, there were only mild effects on chromatin organization. This suggested to us that the rescue effects of HDAC inhibition were independent of the association between chromatin and lamia.

Conclusions

The main conclusions of paper III are:

Threshold for Persistent Activation: vCAFs require 8 days of continous exposure to stiff (25 kPa) environments, together with stiffness and time dependent secreted factors to transition into persistently active myofibroblastic states.

Two Parallel Mechanotransduction Pathways: Two distinct mechanotransduction pathways linking the ECM to chromatin has been identified. One pathway is β_1 integrin-mediated lamin organization dependent, whereas the other is mediated by the formin mDia2 and is independent of changes in lamin organization, likely through its role in assembling actin in the nucleus. Both pathways converge on altering chromatin-lamin interactions.

Epigenetic Encoding of Mechanical Memory: Inhibition of either β_1 integrins or mDia2 pathway prevents persistent activation, but this effect can be rescued by treatment with the histone deacetylase inhibitor TSA. This suggests that chromatin modifications encode the cell's "mechanical memory,"

General discussion

Fibroblasts continuously monitor and respond to changes in their ECM, enabling tissues to adapt during development, wound healing and disease[190, 191]. While FA mediated mechanosensing has been extensively studied, it is still not completely known how cells discriminate between distinct ECM properties with such sensitivity and specificity. In particular, it remains unclear whether features such as the orientational order and alignment of FA components, actively contribute to mechanosensing and how these structural properties encode information about the physical environment. Collectively, the findings presented in this thesis suggest that mechanosensing is not just governed by protein composition and recruitment but also by the dynamic organization and ordering of these proteins. Together, these findings reveal that information from the ECM is progressively organized, amplified and stabilized through changes in FA architecture, cytoskeletal organization and chromatin state. This multiscale framework provides a more integrated understanding of how fibroblasts convert mechanical stimuli into both immediate and long-term phenotypic changes.

At the cell surface, FAs represent the primary site where fibroblasts interact with the ECM[80, 81]. While it has been shown that FAs recruit mechanosensitive proteins in response to force, our findings demonstrate that their internal molecular organization itself constitute an important regulatory mechanism. We show that proteins within FAs, integrins and F-actin, exhibit ECM-dependent orientational ordering that changes in response to subtle alterations in ECM density. This suggests that cells can fine tune their sensitivity to external mechanical cues just through structural reorganizations. Such an organizational mechanism is attractive because many mechanosensitive proteins respond not only to the magnitude of force but also the direction of force[98]. Changes in molecular alignment therefore have the potential to regulate force transmission, protein conformation and downstream signalling that require relatively low energetic investments compared to FA assembly and disassembly. This idea is consistent with observations in other force bearing cytoskeletal systems such as sarcomeres, where actin binding proteins contribute to the molecular ordering of F-actin during sarcomere maturation[192].

Our results further suggest that this organization is dynamically regulated by multiple force generating systems. Actin polymerization appears to establish orientational order, whereas myosin II contractility enhances this organization under conditions requiring greater force transmission. These two force regimes likely

enable fibroblasts to remain highly responsive across a wide range of ECM environments. More broadly these findings emphasize that force generation and structural organization are inseparable components of mechanosensing, where cytoskeletal forces actively organize adhesion architecture, thereby influencing how mechanical information is interpreted[91, 94, 97].

The second study extends this concept of structural regulation by demonstrating that mechanosensing is further refined through functional specialization of FA subpopulations. We identify septin 7 as an important FA associated protein that assembles into distinct structures localized to either peripheral or perinuclear FAs, in an ECM dependent manner. These structurally distinct septin 7 assemblies regulate different aspects of fibroblast behaviour, with peripheral septin 7 primarily controlling sensitivity to ECM density while perinuclear septin 7 promotes adhesion maturation and ECM stiffness sensing. Interestingly, previous studies have reported opposing roles for septins in FA dynamics with some demonstrating that septins promote FA maturation[193] while others suggest they facilitate FA disassembly[194]. Since our findings show that septin 7 performs different functions within different FA subpopulations, septin 7 and probably other septins, may regulate FA dynamics in a context dependent manner with its function determined by the specific adhesion population. These finding reveal that FA heterogeneity extends beyond changes in protein composition and instead involve higher order cytoskeletal organization that enables distinct adhesion populations to perform specialized mechanosensory functions.

While these two studies focus on rapid cellular responses to ECM properties, the final study demonstrates how prolonged mechanical stimulation becomes converted into stable changes in cell identity. We show that sustained exposure to stiff ECMs induce a transition from transient to persistent myofibroblast activation through mechanotransduction pathways that ultimately regulate chromatin organization. Mechanistically, we identify two complementary pathways linking ECM mechanics to chromatin regulation. One pathway depends on β_1 integrin signalling and nuclear lamin organisation, whereas the other requires the formin mDia2, likely through its role in promoting nuclear actin assembly independently of lamin reorganisation. Both pathways ultimately converge on regulating chromatin–lamin interactions. Rescue of persistent activation through inhibition of HDACs further demonstrates that chromatin state represents a principle downstream effector of these mechanotransduction pathways, as also observed by previous studies[172, 173, 181].

Notably, persistent activation is maintained even in the absence of chromatin repositioning and ECM dependent mechanotransduction, highlighting chromatin modifications as key downstream mediators of this activation switch. In agreement with this, our findings show that integrin inhibition disrupts transient activation while also wrinkling the lamina. This response is mechanistically distinct from the effect of TSA and mDia2 which impair activation without altering lamina

morphology. These observations suggest that alterations in chromatin organization and epigenetic modifications function downstream of mechanotransduction signalling to stabilise persistently activated states.

In the context of fibrosis, HATs have been proposed to enhance chromatin accessibility, facilitating the expression of pro-fibrotic genes while HDACs promote chromatin compaction and transcriptional repression of profibrotic loci[195]. Our findings broadly support this paradigm while also revealing a context dependent role for HDACs. TSA attenuated persistent activation, consistent with previous observations that global chromatin decondensation can disrupt gene specific transcriptional programs by compromising the segregation of transcriptionally active and inactive chromatin domains[172]. In contrast, when β1 integrin signalling or mDia2 activity was disrupted, we propose that prolonged culture on stiff substrates failed to establish the chromatin landscape required to sustain persistent activation, resulting in aberrantly elevated HDAC activity. This interpretation is supported by studies that show that integrin engagement can influence HDAC localization and enzymatic activity[184]. Consequently, excessive histone deacetylation epigenetically represses fibroblast activation associated loci, whereas TSA restores acetylation at these regions to rescue persistent activation phenotype without reestablishing lamin-chromatin interactions. Collectively these findings identify histone deacetylation as a downstream effector of both β1 integrin and mDia2 signalling pathways and demonstrate that persistent myofibroblast activation is encoded primarily through epigenetic chromatin state.

Together these findings suggest a model in which structural changes at the cell surface are progressively transmitted through the cytoskeleton to the nucleus, where they become stabilized as epigenetic modifications that maintain fibroblast activation independently of continued mechanical stimulation.

Conclusions

This thesis demonstrates that fibroblast mechanosensing operates through multiple, distinct but complementary mechanisms spanning FAs, cytoskeleton and nucleus. ECM ligand density is sensed primarily through nanoscale reorganization of FA components, where increasing ligand density promotes the orientational alignment of integrins and F-actin, strengthening integrin–ECM interactions through directional catch-bond behaviour. In contrast, substrate stiffness is detected predominantly through force-dependent mechanisms involving actomyosin contractility, driving focal adhesion maturation and mechanotransduction. Importantly, these findings demonstrate that fibroblasts discriminate between different mechanical properties of the ECM using specialised sensing mechanisms rather than a universal response to mechanical stimuli.

The work further demonstrates that FAs are not functionally uniform structures but consist of spatially distinct subpopulations that make unique contributions to mechanosensing. Septin-7 emerged as a critical regulator of this organisation, localising in spatially and temporally distinct patterns in different FA sub-populations. Through its regulation of FA dynamics, septin-7 supports sustained force transmission, efficient mechanosensing and ECM remodelling, highlighting the importance of adhesion heterogeneity in coordinating fibroblast responses to their mechanical environment.

Finally, a mechanistic connection between extracellular mechanical cues and long-term regulation of gene expression is shown. Mechanical signalling through FAs extends beyond cytoskeletal remodelling to influence nuclear architecture to ultimately regulate chromatin–lamin interactions, providing a mechanism by which extracellular mechanical signals can generate persistent epigenetic changes and mechanical memory.

Overall, this thesis advances our understanding of fibroblast mechanobiology by demonstrating that mechanosensing is organised across multiple scales. Specifically, our work identifies three levels of regulation from the nanoscale alignment of FA proteins to the microscale organization of specialized adhesion subpopulations, and the long term nuclear and chromatin remodelling that establishes persistent fibroblast activation. Together, these findings provide an integrated framework for understanding how fibroblasts sense, distinguish and respond to diverse mechanical cues within the extracellular matrix, and how these

signals are converted into stable changes in cell behaviour. These insights have broad implications for physiological processes such as tissue repair and wound healing, as well as pathological conditions including fibrosis, cancer and other mechanically driven diseases.

Limitations and future perspectives

All experiments were performed as 2D *in vitro* studies with cells cultured on fibronectin coated glass or hydrogels with controlled ligand densities and substrate stiffnesses. While these models work well for isolating the effect of individual ECM parameters, they do not capture the complex biochemical or mechanical natures of native or tumour ECMs where cells are simultaneously exposed to heterogenous ECM compositions, fibre architecture, viscoelasticity and dynamic forces that are constantly changing and evolving. Paper I identifies orientational order as an FA architectural feature that contributes to ECM sensing and paper II identifies Sept-7 as a FA regulator, however, whether similar changes in orientational order and septin-7 organization occur in 3D matrices where multiple ECM cues act together should be determined. As 3D hydrogels are not compatible with TIRF microscopy, alternate methods will need to be used such as Time-Resolved Fluorescence Anisotropy Imaging.

In paper I, a correlation between integrin activation and changes in the orientational order of integrins and actin is established, however, the molecular mechanisms for generating and maintaining this organization is unclear. As we could not manipulate orientational order independently of integrin activation, we could not determine whether orientational order is a regulator or a downstream consequence of mechanotransduction. Developing molecular tools capable of selectively perturbing orientational order would enable a more direct assessment of causality.

While Paper II demonstrates that integrin activation controls septin-7 localization at different FA populations, the downstream signalling pathways linking integrins to septins remains unclear. Identifying its interactions with other FA proteins may reveal broader regulatory networks governing cell-ECM signalling and provide mechanistic insights into how septin mediated mechanosensing networks vary across cell states.

Paper I and paper II focus on short term mechanosensing and acute responses while the consequences of these nanoscale organizational changes for long term cell states such as persistent fibroblast activation is unclear. Epigenomic profiling and chromatin accessibility assays during long term culture along with perturbations that disrupt integrin orientation and septin orientation can help determine whether integrin orientation and septin reorganization is maintained and their downstream transcriptional reprogramming pathways.

In paper III, we find that cells require stiffness induced autocrine factors to maintain persistent activation, however, the exact mediators are unknown. Due to the size of our hydrogel system, sufficient volume of conditioned media could not be obtained for mass spectrometry. An upscaled version of the hydrogel system would have to be used for conditioned media proteomics and follow up with targeted depletion assays to establish causality for individual signalling molecules in maintaining persistent CAF activation. Another limitation is that the study does not resolve how mechanotransduction pathways evolve over time to distinguish transient and persistent integrin signalling. While β1 integrin engagement initiates the response, the downstream effectors that stabilize long term activation is unknown. This can be addressed through live cell imaging of FA dynamics and systemic perturbations of candidate pathways at time points. This would help clarify how sustained mechanical inputs are converted into stable transcriptional programs.

Finally, in paper III, histone deacetylation and chromatin-lamin interactions are implicated in persistent activations, but the specific genomic regions involved are not identified. Epigenetic profiling approaches such as ATAC-seq and CUT & RUN can be helpful to map changes in chromatin accessibility and help link mechanical cues to locus specific transcriptional regulation driving fibroblast memory.

Acknowledgments

This thesis would not have been possible without the help and support of the many wonderful people I met along the way. It definitely was not a solo endeavour, and it is a pleasure to thank those who made it possible for me.

I would like to express my gratitude to my supervisor **Vinay Swaminathan** for his guidance, encouragement, and support throughout my PhD, and for contributing almost as many terrible jokes as helpful comments. Thank you for always pushing me to be a better researcher and for your patience during the ups and downs of this journey. You always had new ideas and suggestions to keep us going whenever we were stuck. Whether we were discussing experiments or looking at the thrips eating my office plants under the microscope, your enthusiasm for science rubs off on all of us. Working with you has been a privilege, and my PhD would have been far less memorable without you.

Pontus Nordenfelt, my co-supervisor, thank you for all your support and encouragement. You were always approachable and gave me great input on all my projects. I am especially grateful for your support when I needed extra time to finish my PhD. It has been a pleasure having you as a co-supervisor.

I would like to thank everyone, past and present members, at LCMM. **Maria Allhorn**, you were the best project supervisor anyone could ask for and working together during my first time in the lab is what really made me want to stay. Even after you left the lab, you were still happy to help whenever I came by, which was probably more times than I should have. **Kabilan**, I've enjoyed all our daily chats and laughs, and I always look forward to your endless soap-opera-like life stories. You're a great friend and scientist, and I'm grateful for your help in the lab and your willingness to share your experience and advice. **Linn**, going through this journey together and reminding each other that we'll get there one day made all the difference. I'm glad I got to meet you here. **Valeriia**, working with you during the anisotropy project was the only thing that stopped me from quitting. Thank you for all your help and good luck in Finland! **Zhimeng**, you were always ready to help me and I'm grateful for that. Thanks for all the delicious food you've 'cooked' for us. Finally, a special thanks to **Wesley**. There's never a dull moment when you're around. You've become one of my closest friends and our friendship is one of the best things to come out of these past few years. Thanks for all your help and for always putting up with me.

I would like to thank everyone who was on **D14** before the big move and the new friends on **D11**. I would also like to thank all the members of the **Nordenfelt group**. It's been great working with all of you and thank you for all the help along the way.

I would also like to thank **Sebastian Wasserstrom** and **John Stegmayr** at LBIC for their help with the microscopes. It would have been impossible without them. A special thanks to **Susanne André** and **Anette Saltin** for keeping it all running smoothly.

I'm thankful to all my friends for supporting me along the way. There are more people to thank than I can name individually but I hope you know how much your support and friendships mean to me. Special mentions to **RJ**, **Anki**, and **Shivani**, for your encouragement and laughter, and for always being there through both the good times and the difficult ones. **Josefine**, I'm truly grateful to have met you here. Your friendship has meant so much to me and I couldn't have asked for a better friend. **Vibha**, you're one of the kindest people I know and I'm so glad what started as a work friendship has become so much more. **Aleksandra**, **Phil**, and **Christine**, thank you for all the 'weekly' dinners and the delightfully unhinged conversations.

I would like to thank my family for all their support and encouragement throughout this journey. To my **dad**, thank you for always believing in me and having confidence that I could do this. To my brother, Swarup, thank you for always being in my corner. And to all my **aunts**, **uncles**, and **cousins**: thank you for your endless encouragement and for continuing to ask exactly what it is that I do, even if my explanations have only made it more confusing. I am lucky to have you all in my life.

Finally, to **Oscar**, thank you for all your love, encouragement, and constant support throughout this journey. Thank you for celebrating every success, no matter how small, and for making even the most stressful days lighter. I am so grateful to have you by my side, and I can't wait to see where life takes us next.

References

1. Bakhshandeh, B., et al., Recent progress in the manipulation of biochemical and biophysical cues for engineering functional tissues. Bioeng Transl Med, 2023. **8**(2): p. e10383.
2. Bukoreshtliev, N.V., K. Haase, and A.E. Pelling, Mechanical cues in cellular signalling and communication. Cell Tissue Res, 2013. **352**(1): p. 77-94.
3. Kim, T.J., Mechanobiology: A New Frontier in Biology. Biology (Basel), 2021. **10**(7).
4. Huang, G., Mechanobiology in wound healing. Biophys J, 2022. **121**(2): p. 173-174.
5. Chen, M.B., et al., Mechanobiology in oncology: basic concepts and clinical prospects. Front Cell Dev Biol, 2023. **11**: p. 1239749.
6. Walma, D.A.C. and K.M. Yamada, The extracellular matrix in development. Development, 2020. **147**(10).
7. Karamanos, N.K., et al., A guide to the composition and functions of the extracellular matrix. FEBS J, 2021. **288**(24): p. 6850-6912.
8. Naba, A., Mechanisms of assembly and remodelling of the extracellular matrix. Nat Rev Mol Cell Biol, 2024. **25**(11): p. 865-885.
9. Sun, B., The mechanics of fibrillar collagen extracellular matrix. Cell Rep Phys Sci, 2021. **2**(8).
10. LeBleu, V.S., B. Macdonald, and R. Kalluri, Structure and function of basement membranes. Exp Biol Med (Maywood), 2007. **232**(9): p. 1121-9.
11. Klotzsch, E., et al., Fibronectin forms the most extensible biological fibers displaying switchable force-exposed cryptic binding sites. Proc Natl Acad Sci U S A, 2009. **106**(43): p. 18267-72.
12. Henao-Murillo, L., et al., The Relationship Between Proteoglycan Loss, Overloading-Induced Collagen Damage, and Cyclic Loading in Articular Cartilage. Cartilage, 2021. **13**(2_suppl): p. 1501S-1512S.
13. Nishio, T., et al., Tenascin-C regulates proliferation and migration of cultured astrocytes in a scratch wound assay. Neuroscience, 2005. **132**(1): p. 87-102.
14. SenGupta, S., C.A. Parent, and J.E. Bear, The principles of directed cell migration. Nat Rev Mol Cell Biol, 2021. **22**(8): p. 529-547.
15. Verma, B.K., et al., Substrate Stiffness Modulates TGF-beta Activation and ECM-Associated Gene Expression in Fibroblasts. Bioengineering (Basel), 2023. **10**(9).
16. Wipff, P.J., et al., Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. J Cell Biol, 2007. **179**(6): p. 1311-23.

17. Provenzano, P.P., et al., Collagen reorganization at the tumor-stromal interface facilitates local invasion. BMC Med, 2006. **4**(1): p. 38.
18. Xi, G., et al., Large-scale tumor-associated collagen signatures identify high-risk breast cancer patients. Theranostics, 2021. **11**(7): p. 3229-3243.
19. Petroll, W.M., et al., Corneal Fibroblast Migration Patterns During Intrastromal Wound Healing Correlate With ECM Structure and Alignment. Invest Ophthalmol Vis Sci, 2015. **56**(12): p. 7352-61.
20. Wood, A. and P. Thorogood, An analysis of in vivo cell migration during teleost fin morphogenesis. J Cell Sci, 1984. **66**: p. 205-22.
21. Gerard, C. and A. Goldbeter, The balance between cell cycle arrest and cell proliferation: control by the extracellular matrix and by contact inhibition. Interface Focus, 2014. **4**(3): p. 20130075.
22. Solomonov, I., O. Kollet, and I. Sagi, Extracellular matrix and proteolysis: mechanisms driving irreversible changes and shaping cell behavior. FEBS J, 2025.
23. Yao, Z., et al., Tissue transglutaminase: a multifunctional and multisite regulator in health and disease. Physiol Rev, 2024. **104**(1): p. 281-325.
24. Bathish, B., et al., Peroxidasin mediates bromination of tyrosine residues in the extracellular matrix. J Biol Chem, 2020. **295**(36): p. 12697-12705.
25. Iozzo, R.V. and M.A. Gubbiotti, Extracellular matrix: The driving force of mammalian diseases. Matrix Biol, 2018. **71-72**: p. 1-9.
26. Mixon, A., A.S. Bahar-Moni, and T.R. Faisal, Mechanical characterization of articular cartilage degraded combinedly with MMP-1 and MMP-9. J Mech Behav Biomed Mater, 2022. **129**: p. 105131.
27. Serraino, G.F., et al., Metalloproteinases and Hypertrophic Cardiomyopathy: A Systematic Review. Biomolecules, 2023. **13**(4).
28. Wong, Z.Y., et al., Why does understanding the biology of fibroblasts in immunity really matter? PLoS Biol, 2023. **21**(2): p. e3001954.
29. Plikus, M.V., et al., Fibroblasts: Origins, definitions, and functions in health and disease. Cell, 2021. **184**(15): p. 3852-3872.
30. Younesi, F.S., et al., Fibroblast and myofibroblast activation in normal tissue repair and fibrosis. Nat Rev Mol Cell Biol, 2024. **25**(8): p. 617-638.
31. Talbott, H.E., et al., Wound healing, fibroblast heterogeneity, and fibrosis. Cell Stem Cell, 2022. **29**(8): p. 1161-1180.
32. Shameem, M., et al., Cardiac Fibroblasts: Helping or Hurting. Genes (Basel), 2025. **16**(4).
33. Jacobs, M.E., et al., Endothelial to mesenchymal transition in kidney fibrosis. Nephrol Dial Transplant, 2024. **39**(5): p. 752-760.
34. Sahai, E., et al., A framework for advancing our understanding of cancer-associated fibroblasts. Nat Rev Cancer, 2020. **20**(3): p. 174-186.
35. Desbois, M. and Y. Wang, Cancer-associated fibroblasts: Key players in shaping the tumor immune microenvironment. Immunol Rev, 2021. **302**(1): p. 241-258.

36. Gunaydin, G., S.A. Kesikli, and D. Guc, Cancer associated fibroblasts have phenotypic and functional characteristics similar to the fibrocytes that represent a novel MDSC subset. Oncoimmunology, 2015. **4**(9): p. e1034918.
37. Linxweiler, J., et al., Cancer-associated fibroblasts stimulate primary tumor growth and metastatic spread in an orthotopic prostate cancer xenograft model. Sci Rep, 2020. **10**(1): p. 12575.
38. Rupert, J., et al., Depletion of Adipose Stroma-Like Cancer-Associated Fibroblasts Potentiates Pancreatic Cancer Immunotherapy. Cancer Res Commun, 2025. **5**(1): p. 5-12.
39. Jungwirth, U., et al., Impairment of a distinct cancer-associated fibroblast population limits tumour growth and metastasis. Nat Commun, 2021. **12**(1): p. 3516.
40. Pang, N., et al., Cancer-associated fibroblasts barrier breaking via TGF-beta blockade paved way for docetaxel micelles delivery to treat pancreatic cancer. Int J Pharm, 2024. **665**: p. 124706.
41. Jenkins, L., et al., Cancer-Associated Fibroblasts Suppress CD8+ T-cell Infiltration and Confer Resistance to Immune-Checkpoint Blockade. Cancer Res, 2022. **82**(16): p. 2904-2917.
42. Rhim, A.D., et al., Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. Cancer Cell, 2014. **25**(6): p. 735-47.
43. Ozdemir, B.C., et al., Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. Cancer Cell, 2014. **25**(6): p. 719-34.
44. Biffi, G., et al., IL1-Induced JAK/STAT Signaling Is Antagonized by TGFbeta to Shape CAF Heterogeneity in Pancreatic Ductal Adenocarcinoma. Cancer Discov, 2019. **9**(2): p. 282-301.
45. Goulet, C.R., et al., Cancer-associated fibroblasts induce epithelial-mesenchymal transition of bladder cancer cells through paracrine IL-6 signalling. BMC Cancer, 2019. **19**(1): p. 137.
46. Chen, S., et al., Cancer-associated fibroblast-induced M2-polarized macrophages promote hepatocellular carcinoma progression via the plasminogen activator inhibitor-1 pathway. Int J Oncol, 2021. **59**(2).
47. Gao, D., et al., Microenvironmental regulation in tumor progression: Interactions between cancer-associated fibroblasts and immune cells. Biomed Pharmacother, 2023. **167**: p. 115622.
48. Huang, H., et al., Mesothelial cell-derived antigen-presenting cancer-associated fibroblasts induce expansion of regulatory T cells in pancreatic cancer. Cancer Cell, 2022. **40**(6): p. 656-673 e7.
49. Sousa, C.M., et al., Pancreatic stellate cells support tumour metabolism through autophagic alanine secretion. Nature, 2016. **536**(7617): p. 479-83.
50. Zhang, Z., Q. Zhang, and Y. Wang, CAF-mediated tumor vascularization: From mechanistic insights to targeted therapies. Cell Signal, 2025. **132**: p. 111827.
51. Mizutani, Y., et al., Meflin-Positive Cancer-Associated Fibroblasts Inhibit Pancreatic Carcinogenesis. Cancer Res, 2019. **79**(20): p. 5367-5381.

52. Nissen, N.I., M. Karsdal, and N. Willumsen, Collagens and Cancer associated fibroblasts in the reactive stroma and its relation to Cancer biology. J Exp Clin Cancer Res, 2019. **38**(1): p. 115.
53. Zhang, M. and B. Zhang, Extracellular matrix stiffness: mechanisms in tumor progression and therapeutic potential in cancer. Exp Hematol Oncol, 2025. **14**(1): p. 54.
54. Lachowski, D., et al., Matrix stiffness modulates the activity of MMP-9 and TIMP-1 in hepatic stellate cells to perpetuate fibrosis. Sci Rep, 2019. **9**(1): p. 7299.
55. Whatcott, C.J., et al., Inhibition of ROCK1 kinase modulates both tumor cells and stromal fibroblasts in pancreatic cancer. PLoS One, 2017. **12**(8): p. e0183871.
56. Erdogan, B., et al., Cancer-associated fibroblasts promote directional cancer cell migration by aligning fibronectin. J Cell Biol, 2017. **216**(11): p. 3799-3816.
57. Graham, J., M. Raghunath, and V. Vogel, Fibrillar fibronectin plays a key role as nucleator of collagen I polymerization during macromolecular crowding-enhanced matrix assembly. Biomater Sci, 2019. **7**(11): p. 4519-4535.
58. Kii, I., et al., Incorporation of tenascin-C into the extracellular matrix by periostin underlies an extracellular meshwork architecture. J Biol Chem, 2010. **285**(3): p. 2028-39.
59. Neves, M.I., et al., Glycosaminoglycan-Inspired Biomaterials for the Development of Bioactive Hydrogel Networks. Molecules, 2020. **25**(4).
60. Hiemer, S.E., A.D. Szymaniak, and X. Varelas, The transcriptional regulators TAZ and YAP direct transforming growth factor beta-induced tumorigenic phenotypes in breast cancer cells. J Biol Chem, 2014. **289**(19): p. 13461-74.
61. Wang, C., et al., MicroRNA-10 Family Promotes the Epithelial-to-Mesenchymal Transition in Renal Fibrosis by the PTEN/Akt Pathway. Curr Issues Mol Biol, 2022. **44**(12): p. 6059-6074.
62. Duangrat, R., W. Parichatikanond, and S. Mangmool, Dual Blockade of TGF-beta Receptor and Endothelin Receptor Synergistically Inhibits Angiotensin II-Induced Myofibroblast Differentiation: Role of AT(1)R/G(alphaq)-Mediated TGF-beta1 and ET-1 Signaling. Int J Mol Sci, 2023. **24**(8).
63. Hammer, A.M., et al., Stromal PDGFR-alpha Activation Enhances Matrix Stiffness, Impedes Mammary Ductal Development, and Accelerates Tumor Growth. Neoplasia, 2017. **19**(6): p. 496-508.
64. Pilling, D., et al., TNF-alpha-stimulated fibroblasts secrete lumican to promote fibrocyte differentiation. Proc Natl Acad Sci U S A, 2015. **112**(38): p. 11929-34.
65. Nam, S., et al., Strain-enhanced stress relaxation impacts nonlinear elasticity in collagen gels. Proc Natl Acad Sci U S A, 2016. **113**(20): p. 5492-7.
66. Zhang, Y., et al., HIF-1alpha is necessary for activation and tumour-promotion effect of cancer-associated fibroblasts in lung cancer. J Cell Mol Med, 2021. **25**(12): p. 5457-5469.
67. Goldmann, W.H., Mechanotransduction and focal adhesions. Cell Biol Int, 2012. **36**(7): p. 649-52.

68. Kuo, J.C., Mechanotransduction at focal adhesions: integrating cytoskeletal mechanics in migrating cells. J Cell Mol Med, 2013. **17**(6): p. 704-12.
69. Leitinger, B., et al., The regulation of integrin function by Ca(2+). Biochim Biophys Acta, 2000. **1498**(2-3): p. 91-8.
70. Takada, Y., X. Ye, and S. Simon, The integrins. Genome Biol, 2007. **8**(5): p. 215.
71. Pan, L., et al., Research advances on structure and biological functions of integrins. Springerplus, 2016. **5**(1): p. 1094.
72. Luo, B.H. and T.A. Springer, Integrin structures and conformational signaling. Curr Opin Cell Biol, 2006. **18**(5): p. 579-86.
73. Wang, J.H., Pull and push: talin activation for integrin signaling. Cell Res, 2012. **22**(11): p. 1512-4.
74. Zhu, L., et al., Structure of Rap1b bound to talin reveals a pathway for triggering integrin activation. Nat Commun, 2017. **8**(1): p. 1744.
75. Luo, B.H., C.V. Carman, and T.A. Springer, Structural basis of integrin regulation and signaling. Annu Rev Immunol, 2007. **25**: p. 619-47.
76. Njus, B.H., et al., Conformational mAb as a tool for integrin ligand discovery. Assay Drug Dev Technol, 2009. **7**(5): p. 507-15.
77. Iwamoto, D.V. and D.A. Calderwood, Regulation of integrin-mediated adhesions. Curr Opin Cell Biol, 2015. **36**: p. 41-7.
78. Wehrle-Haller, B. and B.A. Imhof, Integrin-dependent pathologies. J Pathol, 2003. **200**(4): p. 481-7.
79. Chastney, M.R., J.R.W. Conway, and J. Ivaska, Integrin adhesion complexes. Curr Biol, 2021. **31**(10): p. R536-R542.
80. Burridge, K., Focal adhesions: a personal perspective on a half century of progress. FEBS J, 2017. **284**(20): p. 3355-3361.
81. Legerstee, K. and A.B. Houtsmuller, A Layered View on Focal Adhesions. Biology (Basel), 2021. **10**(11).
82. Wehrle-Haller, B., Structure and function of focal adhesions. Curr Opin Cell Biol, 2012. **24**(1): p. 116-24.
83. Petit, V. and J.P. Thiery, Focal adhesions: structure and dynamics. Biol Cell, 2000. **92**(7): p. 477-94.
84. Kanchanawong, P., et al., Nanoscale architecture of integrin-based cell adhesions. Nature, 2010. **468**(7323): p. 580-4.
85. Goult, B.T., N.H. Brown, and M.A. Schwartz, Talin in mechanotransduction and mechanomemory at a glance. J Cell Sci, 2021. **134**(20).
86. Goldmann, W.H., Role of vinculin in cellular mechanotransduction. Cell Biol Int, 2016. **40**(3): p. 241-56.
87. Vicente-Manzanares, M. and A.R. Horwitz, Adhesion dynamics at a glance. J Cell Sci, 2011. **124**(Pt 23): p. 3923-7.
88. Katsuta, H., M. Sokabe, and H. Hirata, From stress fiber to focal adhesion: a role of actin crosslinkers in force transmission. Front Cell Dev Biol, 2024. **12**: p. 1444827.

89. Sun, Z., S.S. Guo, and R. Fassler, Integrin-mediated mechanotransduction. J Cell Biol, 2016. **215**(4): p. 445-456.
90. Kong, F., et al., Demonstration of catch bonds between an integrin and its ligand. J Cell Biol, 2009. **185**(7): p. 1275-84.
91. Yao, M., et al., The mechanical response of talin. Nat Commun, 2016. **7**: p. 11966.
92. Owen, L.M., et al., The C-terminal actin-binding domain of talin forms an asymmetric catch bond with F-actin. Proc Natl Acad Sci U S A, 2022. **119**(10): p. e2109329119.
93. Pena Ccoa, W.J., et al., A direct computational assessment of vinculin-actin unbinding kinetics reveals catch-bonding behavior. Proc Natl Acad Sci U S A, 2025. **122**(21): p. e2425982122.
94. Huang, D.L., et al., Vinculin forms a directionally asymmetric catch bond with F-actin. Science, 2017. **357**(6352): p. 703-706.
95. Kluger, C., et al., Different Vinculin Binding Sites Use the Same Mechanism to Regulate Directional Force Transduction. Biophys J, 2020. **118**(6): p. 1344-1356.
96. Golji, J. and M.R.K. Mofrad, The talin dimer structure orientation is mechanically regulated. Biophys J, 2014. **107**(8): p. 1802-1809.
97. Swaminathan, V., et al., Actin retrograde flow actively aligns and orients ligand-engaged integrins in focal adhesions. Proc Natl Acad Sci U S A, 2017. **114**(40): p. 10648-10653.
98. Swaminathan, V. and M. Gloerich, Decoding mechanical cues by molecular mechanotransduction. Curr Opin Cell Biol, 2021. **72**: p. 72-80.
99. Ciobanasu, C., B. Faivre, and C. Le Clainche, Actin dynamics associated with focal adhesions. Int J Cell Biol, 2012. **2012**: p. 941292.
100. Pasapera, A.M., et al., Rac1-dependent phosphorylation and focal adhesion recruitment of myosin IIA regulates migration and mechanosensing. Curr Biol, 2015. **25**(2): p. 175-186.
101. Vicente-Manzanares, M., et al., Segregation and activation of myosin IIB creates a rear in migrating cells. J Cell Biol, 2008. **183**(3): p. 543-54.
102. Rotty, J.D., C. Wu, and J.E. Bear, New insights into the regulation and cellular functions of the ARP2/3 complex. Nat Rev Mol Cell Biol, 2013. **14**(1): p. 7-12.
103. Valencia, D.A. and M.E. Quinlan, Formins. Curr Biol, 2021. **31**(10): p. R517-R522.
104. Seetharaman, S. and S. Etienne-Manneville, Microtubules at focal adhesions - a double-edged sword. J Cell Sci, 2019. **132**(19).
105. Leube, R.E., M. Moch, and R. Windoffer, Intermediate filaments and the regulation of focal adhesion. Curr Opin Cell Biol, 2015. **32**: p. 13-20.
106. Mostowy, S. and P. Cossart, Septins: the fourth component of the cytoskeleton. Nat Rev Mol Cell Biol, 2012. **13**(3): p. 183-94.
107. Spiliotis, E.T. and K. Nakos, Cellular functions of actin- and microtubule-associated septins. Curr Biol, 2021. **31**(10): p. R651-R666.
108. Yeh, Y.T., et al., Matrix stiffness regulates endothelial cell proliferation through septin 9. PLoS One, 2012. **7**(10): p. e46889.

109. Kremer, B.E., L.A. Adang, and I.G. Macara, Septins regulate actin organization and cell-cycle arrest through nuclear accumulation of NCK mediated by SOCS7. Cell, 2007. **130**(5): p. 837-50.
110. Kinoshita, M., The septins. Genome Biol, 2003. **4**(11): p. 236.
111. Lam, M. and F. Calvo, Regulation of mechanotransduction: Emerging roles for septins. Cytoskeleton (Hoboken), 2019. **76**(1): p. 115-122.
112. Mitra, S.K. and D.D. Schlaepfer, Integrin-regulated FAK-Src signaling in normal and cancer cells. Curr Opin Cell Biol, 2006. **18**(5): p. 516-23.
113. Lawson, C.D. and K. Burridge, The on-off relationship of Rho and Rac during integrin-mediated adhesion and cell migration. Small GTPases, 2014. **5**: p. e27958.
114. Yuan, Y., et al., The Role of the RhoA/ROCK Signaling Pathway in Mechanical Strain-Induced Scleral Myofibroblast Differentiation. Invest Ophthalmol Vis Sci, 2018. **59**(8): p. 3619-3629.
115. Tanimura, S. and K. Takeda, ERK signalling as a regulator of cell motility. J Biochem, 2017. **162**(3): p. 145-154.
116. New, D.C., et al., G protein-coupled receptor-induced Akt activity in cellular proliferation and apoptosis. FEBS J, 2007. **274**(23): p. 6025-36.
117. Guarino, M., Src signaling in cancer invasion. J Cell Physiol, 2010. **223**(1): p. 14-26.
118. Cai, X., K.C. Wang, and Z. Meng, Mechanoregulation of YAP and TAZ in Cellular Homeostasis and Disease Progression. Front Cell Dev Biol, 2021. **9**: p. 673599.
119. Defilippi, P., P. Di Stefano, and S. Cabodi, p130Cas: a versatile scaffold in signaling networks. Trends Cell Biol, 2006. **16**(5): p. 257-63.
120. Gorska, A. and A.J. Mazur, Integrin-linked kinase (ILK): the known vs. the unknown and perspectives. Cell Mol Life Sci, 2022. **79**(2): p. 100.
121. Pennacchio, F.A., et al., Tailoring Cellular Function: The Contribution of the Nucleus in Mechanotransduction. Front Bioeng Biotechnol, 2020. **8**: p. 596746.
122. Niethammer, P., Components and Mechanisms of Nuclear Mechanotransduction. Annu Rev Cell Dev Biol, 2021. **37**: p. 233-256.
123. Bouzid, T., et al., The LINC complex, mechanotransduction, and mesenchymal stem cell function and fate. J Biol Eng, 2019. **13**: p. 68.
124. King, M.C., Dynamic regulation of LINC complex composition and function across tissues and contexts. FEBS Lett, 2023. **597**(22): p. 2823-2832.
125. Vahabikashi, A., et al., Nuclear lamin isoforms differentially contribute to LINC complex-dependent nucleocytoskeletal coupling and whole-cell mechanics. Proc Natl Acad Sci U S A, 2022. **119**(17): p. e2121816119.
126. Yang, T., et al., Role of Emerin in regulating fibroblast differentiation and migration at the substrate of stiffness coupled topology. Acta Biochim Biophys Sin (Shanghai), 2024. **56**(9): p. 1387-1400.
127. Jin, Q., et al., Acute downregulation of emerin alters actomyosin cytoskeleton connectivity and function. Biophys J, 2023. **122**(18): p. 3690-3703.
128. Vahabikashi, A., et al., Nuclear lamins: Structure and function in mechanobiology. APL Bioeng, 2022. **6**(1): p. 011503.

129. Donnaloja, F., et al., Lamin A/C Mechanotransduction in Laminopathies. Cells, 2020. **9**(5).
130. Swift, J., et al., Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation. Science, 2013. **341**(6149): p. 1240104.
131. Wang, T.C., et al., Matrix stiffness drives drop like nuclear deformation and lamin A/C tension-dependent YAP nuclear localization. Nat Commun, 2024. **15**(1): p. 10151.
132. Nazer, E., To be or not be (in the LAD): emerging roles of lamin proteins in transcriptional regulation. Biochem Soc Trans, 2022. **50**(2): p. 1035-1044.
133. Kloc, M. and J. Wosik, Mechanical Forces, Nucleus, Chromosomes, and Chromatin. Biomolecules, 2025. **15**(3).
134. Stephens, A.D., et al., Chromatin histone modifications and rigidity affect nuclear morphology independent of lamins. Mol Biol Cell, 2018. **29**(2): p. 220-233.
135. Stephens, A.D., E.J. Banigan, and J.F. Marko, Chromatin's physical properties shape the nucleus and its functions. Curr Opin Cell Biol, 2019. **58**: p. 76-84.
136. Amar, K., et al., Effects of forces on chromatin. APL Bioeng, 2021. **5**(4): p. 041503.
137. Hsia, C.R., D.P. Melters, and Y. Dalal, The Force is Strong with This Epigenome: Chromatin Structure and Mechanobiology. J Mol Biol, 2023. **435**(11): p. 168019.
138. Matsuda, A. and M.R.K. Mofrad, On the nuclear pore complex and its emerging role in cellular mechanotransduction. APL Bioeng, 2022. **6**(1): p. 011504.
139. Enyedi, B. and P. Niethammer, Nuclear membrane stretch and its role in mechanotransduction. Nucleus, 2017. **8**(2): p. 156-161.
140. Kalukula, Y., et al., Mechanics and functional consequences of nuclear deformations. Nat Rev Mol Cell Biol, 2022. **23**(9): p. 583-602.
141. Visa, N. and P. Percipalle, Nuclear functions of actin. Cold Spring Harb Perspect Biol, 2010. **2**(4): p. a000620.
142. Kukalev, A., et al., Actin and hnRNP U cooperate for productive transcription by RNA polymerase II. Nat Struct Mol Biol, 2005. **12**(3): p. 238-44.
143. Kapoor, P. and X. Shen, Mechanisms of nuclear actin in chromatin-remodeling complexes. Trends Cell Biol, 2014. **24**(4): p. 238-46.
144. Kelpsch, D.J. and T.L. Tootle, Nuclear Actin: From Discovery to Function. Anat Rec (Hoboken), 2018. **301**(12): p. 1999-2013.
145. Davidson, P.M. and B. Cadot, Actin on and around the Nucleus. Trends Cell Biol, 2021. **31**(3): p. 211-223.
146. Schwartz, C., et al., Lamins and nesprin-1 mediate inside-out mechanical coupling in muscle cell precursors through FHOD1. Sci Rep, 2017. **7**(1): p. 1253.
147. Kyheroinen, S. and M.K. Vartiainen, Nuclear actin dynamics in gene expression and genome organization. Semin Cell Dev Biol, 2020. **102**: p. 105-112.
148. Sankaran, J.S., et al., Knockdown of formin mDia2 alters lamin B1 levels and increases osteogenesis in stem cells. Stem Cells, 2020. **38**(1): p. 102-117.

149. Provenzano, P.P. and P.J. Keely, Mechanical signaling through the cytoskeleton regulates cell proliferation by coordinated focal adhesion and Rho GTPase signaling. J Cell Sci, 2011. **124**(Pt 8): p. 1195-205.
150. Sun, Z., A. Lambacher, and R. Fassler, Nascent adhesions: from fluctuations to a hierarchical organization. Curr Biol, 2014. **24**(17): p. R801-3.
151. Stricker, J., et al., Myosin II-mediated focal adhesion maturation is tension insensitive. PLoS One, 2013. **8**(7): p. e70652.
152. Beedle, A.E.M., et al., Fibrillar adhesion dynamics govern the timescales of nuclear mechano-response via the vimentin cytoskeleton. Nat Mater, 2026.
153. Bhadriraju, K., et al., Activation of ROCK by RhoA is regulated by cell adhesion, shape, and cytoskeletal tension. Exp Cell Res, 2007. **313**(16): p. 3616-23.
154. Lachowski, D., et al., FAK controls the mechanical activation of YAP, a transcriptional regulator required for durotaxis. FASEB J, 2018. **32**(2): p. 1099-1107.
155. Dupont, S., Regulation of YAP/TAZ Activity by Mechanical Cues: An Experimental Overview. Methods Mol Biol, 2019. **1893**: p. 183-202.
156. Esnault, C., et al., Rho-actin signaling to the MRTF coactivators dominates the immediate transcriptional response to serum in fibroblasts. Genes Dev, 2014. **28**(9): p. 943-58.
157. Yang, C., et al., Mechanical memory and dosing influence stem cell fate. Nat Mater, 2014. **13**(6): p. 645-52.
158. Nasrollahi, S., et al., Past matrix stiffness primes epithelial cells and regulates their future collective migration through a mechanical memory. Biomaterials, 2017. **146**: p. 146-155.
159. Dudaryeva, O.Y., et al., Implications of Cellular Mechanical Memory in Bioengineering. ACS Biomater Sci Eng, 2023. **9**(11): p. 5985-5998.
160. Mierke, C.T., Impact of the ECM on the Mechanical Memory of Cancer Cells. Cells, 2025. **14**(21).
161. Balestrini, J.L., et al., The mechanical memory of lung myofibroblasts. Integr Biol (Camb), 2012. **4**(4): p. 410-21.
162. Lee, J., A.A. Abdeen, and K.A. Kilian, Rewiring mesenchymal stem cell lineage specification by switching the biophysical microenvironment. Sci Rep, 2014. **4**: p. 5188.
163. Lee, J., et al., Controlling cell geometry on substrates of variable stiffness can tune the degree of osteogenesis in human mesenchymal stem cells. J Mech Behav Biomed Mater, 2014. **38**: p. 209-18.
164. Dunham, C., et al., Adipose stem cells exhibit mechanical memory and reduce fibrotic contracture in a rat elbow injury model. FASEB J, 2020. **34**(9): p. 12976-12990.
165. Frank, V., et al., Frequent mechanical stress suppresses proliferation of mesenchymal stem cells from human bone marrow without loss of multipotency. Sci Rep, 2016. **6**: p. 24264.
166. Hong, Y., et al., Cell-matrix feedback controls stretch-induced cellular memory and fibroblast activation. Proc Natl Acad Sci U S A, 2025. **122**(12): p. e2322762122.

167. Moon, S.Y., et al., Cell contractility drives mechanical memory of oral squamous cell carcinoma. Mol Biol Cell, 2023. **34**(9): p. ar89.
168. Cambria, E., et al., Linking cell mechanical memory and cancer metastasis. Nat Rev Cancer, 2024. **24**(3): p. 216-228.
169. Scott, K.E., S.I. Fraley, and P. Rangamani, A spatial model of YAP/TAZ signaling reveals how stiffness, dimensionality, and shape contribute to emergent outcomes. Proc Natl Acad Sci U S A, 2021. **118**(20).
170. Rashid, F., et al., Mechanomemory after short episodes of intermittent stresses induces YAP translocation via increasing F-actin. APL Bioeng, 2025. **9**(2): p. 026107.
171. Dingal, P.C., et al., Fractal heterogeneity in minimal matrix models of scars modulates stiff-niche stem-cell responses via nuclear exit of a mechanorepressor. Nat Mater, 2015. **14**(9): p. 951-60.
172. Walker, C.J., et al., Nuclear mechanosensing drives chromatin remodelling in persistently activated fibroblasts. Nat Biomed Eng, 2021. **5**(12): p. 1485-1499.
173. Heo, S.J., et al., Biophysical Regulation of Chromatin Architecture Instills a Mechanical Memory in Mesenchymal Stem Cells. Sci Rep, 2015. **5**: p. 16895.
174. Teodoridis, J.M., G. Strathdee, and R. Brown, Epigenetic silencing mediated by CpG island methylation: potential as a therapeutic target and as a biomarker. Drug Resist Updat, 2004. **7**(4-5): p. 267-78.
175. Bhowmick, N.A., et al., TGF-beta signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. Science, 2004. **303**(5659): p. 848-51.
176. Trimboli, A.J., et al., Pten in stromal fibroblasts suppresses mammary epithelial tumours. Nature, 2009. **461**(7267): p. 1084-91.
177. Xiao, Q., et al., Cancer-Associated Fibroblasts in Pancreatic Cancer Are Reprogrammed by Tumor-Induced Alterations in Genomic DNA Methylation. Cancer Res, 2016. **76**(18): p. 5395-404.
178. Thum, T., et al., MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. Nature, 2008. **456**(7224): p. 980-4.
179. van Rooij, E., et al., Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. Proc Natl Acad Sci U S A, 2008. **105**(35): p. 13027-32.
180. Bertero, T., et al., Matrix Remodeling Promotes Pulmonary Hypertension through Feedback Mechanoactivation of the YAP/TAZ-miR-130/301 Circuit. Cell Rep, 2015. **13**(5): p. 1016-32.
181. Killaars, A.R., et al., Extended Exposure to Stiff Microenvironments Leads to Persistent Chromatin Remodeling in Human Mesenchymal Stem Cells. Adv Sci (Weinh), 2019. **6**(3): p. 1801483.
182. Liang, G., et al., Inhibiting stromal Class I HDACs curbs pancreatic cancer progression. Nat Commun, 2023. **14**(1): p. 7791.
183. Jones, D.L., et al., TGFbeta-induced fibroblast activation requires persistent and targeted HDAC-mediated gene repression. J Cell Sci, 2019. **132**(20).

184. Sato, T., et al., A FAK/HDAC5 signaling axis controls osteocyte mechanotransduction. Nat Commun, 2020. **11**(1): p. 3282.
185. Terri, M., et al., HDAC1/2 control mesothelium/ovarian cancer adhesive interactions impacting on Talin-1-alpha5beta1-integrin-mediated actin cytoskeleton and extracellular matrix protein remodeling. J Exp Clin Cancer Res, 2024. **43**(1): p. 27.
186. Cai, J., et al., Deciphering the dynamics: Exploring the impact of mechanical forces on histone acetylation. FASEB J, 2024. **38**(15): p. e23849.
187. Lakins, J.N., A.R. Chin, and V.M. Weaver, Exploring the link between human embryonic stem cell organization and fate using tension-calibrated extracellular matrix functionalized polyacrylamide gels. Methods Mol Biol, 2012. **916**: p. 317-50.
188. Proteintech. IF imaging: Widefield versus confocal microscopy. [Image] 2021 [cited July 21, 2026; Available from: https://www.ptglab.com/news/blog/if-imaging-widefield-versus-confocal-microscopy/?srsltid=AfmBOor3pj39q55KCmAO9TAdnIZjkyNmfWg2kOgizLyWL0CwzmzyMB79.
189. Kisler, K., R.H. Chow, and R. Dominguez, Fluorescently-Labeled Estradiol Internalization and Membrane Trafficking in Live N-38 Neuronal Cells Visualized with Total Internal Reflection Fluorescence Microscopy. J Steroids Horm Sci, 2013. **Suppl 12**.
190. Boraldi, F., et al., The Role of Fibroblasts in Skin Homeostasis and Repair. Biomedicines, 2024. **12**(7).
191. DeLeon-Pennell, K.Y., T.H. Barker, and M.L. Lindsey, Fibroblasts: The arbiters of extracellular matrix remodeling. Matrix Biol, 2020. **91-92**: p. 1-7.
192. Loison, O., et al., Polarization-resolved microscopy reveals a muscle myosin motor-independent mechanism of molecular actin ordering during sarcomere maturation. PLoS Biol, 2018. **16**(4): p. e2004718.
193. Dolat, L., et al., Septins promote stress fiber-mediated maturation of focal adhesions and renal epithelial motility. J Cell Biol, 2014. **207**(2): p. 225-35.
194. Merenich, D., et al., Septins guide noncentrosomal microtubules to promote focal adhesion disassembly in migrating cells. Mol Biol Cell, 2022. **33**(5): p. ar40.
195. Shao, J., J. Liu, and S. Zuo, Roles of Epigenetics in Cardiac Fibroblast Activation and Fibrosis. Cells, 2022. **11**(15).

Printed by Media-Tryck, Lund 2026 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Clinical sciences

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2026:103
ISBN 978-91-8021-901-3
ISSN 1652-8220