Extracellular Matrix and Motor Axon Pathfinding
What Zebrafish Teach Us

Florence Ruggiero

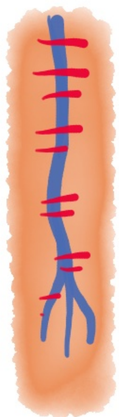
Extracellular Matrix Networks

- tissue specific
- heterogenous
- dynamic

1000 genes

Collagens as key players

Growing neurites follow ColXV-B micropatterned tracks



collagen biosynthesis is a 2-step mechanism involving 2 independent pathways

Shh + MuSK signal

- Transparent

Zebrafish Model System

- Neuromuscular System
- specialised ECMs

- Axon Path ECM

- Mutations can lead to mobility issues

Adaxial cells lay down matrix fingerprint

- ColXV-B

Stacking → Migration

- Axon engulfed in ECM

Role of Sonic Hedgehog

Tenascin C = repulsant

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Basement Membrane Remodelling Regulates Mouse Embryogenesis

The importance of perforations

Christos Kyprianou

Early mouse embryo development

Pre ⟶ Post-implantation

Basement Membrane surrounds egg cylinder during elongation

- maintains egg cylinder shape

Treat embryos with collagenase → changes aspect ratio

Role of Matrix Metalloproteinases ⟶ MMP2 + MMP14 expression Spatiotemporally matches perforations } Nodal drives expression of MMPs

Embryo elongation is perturbed in absence of MMPs

Basement Membrane is perforated

Distribution of perforations changes
Become asymmetrical ⟶

- Relevance to Anterior-Posterior Axis Establishment

Perforation distribution correlates with Anterior-Posterior Axis

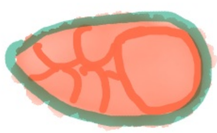
Perturbation of **Nodal** leads to aberrant **Basement Membrane** morphology

"Like a run in a stocking" @ATJCagan

Dynamic Basement Membrane Remodelling for Organ Morphogenesis

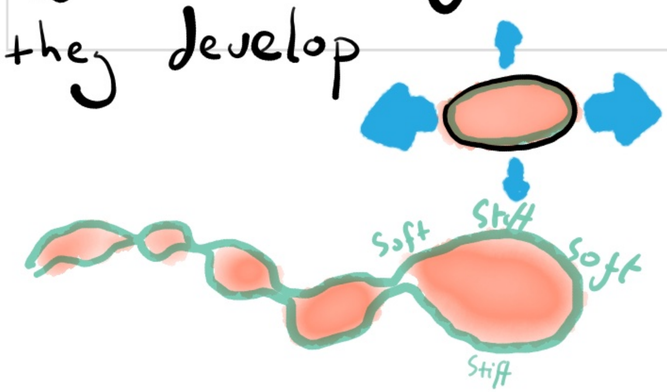
Sally Horne-Badovinac

Basement Membranes are extensively remodeled during development



Drosophila egg chambers

Egg chambers elongate as they develop



Fibrils contain multiple BM proteins

BM Ridges

Follicle stress fibers

Do integrins in stress fibers contribute to polarisation of BM?

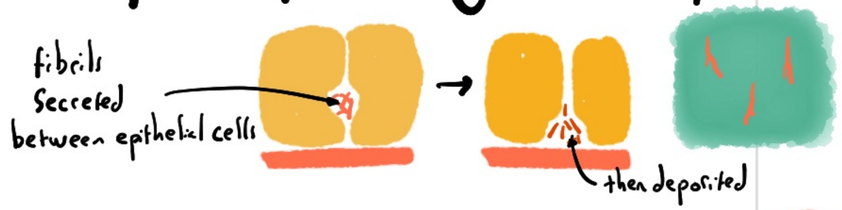
Sliding Adhesions

Fibrils form on a largely isotropic matrix

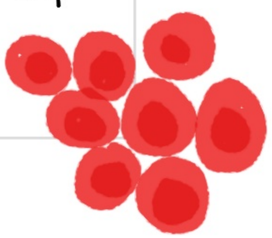
rotation during devo

BM fibrils + ridges

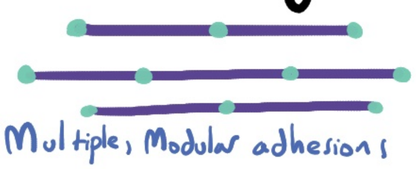
Fibrils formed from newly secreted protein



What is the role of Dystroglycan



Individual stress fibers persist as the cell migrates - "treadmilling"



Multiple, Modular adhesions

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Intracellular Trafficking and Processing of Procollagen

COPII-dependent budding

why collagen most affected cargo on disruption of Golgi apparatus?

How to get to the Golgi-apparatus?

Localization of endogenous procollagen in primary fibroblasts

How is initial transport of Collagen linked to its eventual deposition?

David Stephens

Giantin KO RPE1 cell line by CRISPR

outlining of the Golgi
20 mins = filling of Golgi

Large Structures label with ER markers - not Golgi

COPII-dependent pathway

Early Secretory Pathway

Giantin
- Loss results in ECM defects

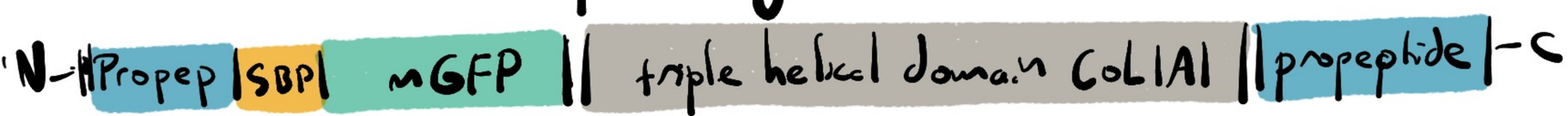
Zebrafish

↑ in fin ray fractures

Collagen processing altered in giantin KO cells
- Causes defect in intracellular processing of procollagen

Intracellular N-terminal processing of COL1A1

A new Controllable procollagen reporter



N-pro cleavage site

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Matrix Secretion, Assembly & Turnover

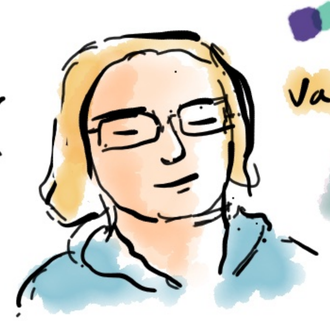
FLASH TALKS!

Jessica Morgner

Lambl Dendra2 - a new mouse model to study basement membrane dynamics in vivo

Study tumor progression in mouse model

- Basement remodelling



Dendra2

Variety of organs

Isabelle Collins

Identification of a novel regulator of ADAMTS-5-mediated aggrecan degradation in chondrocytes

Ciliary proteins

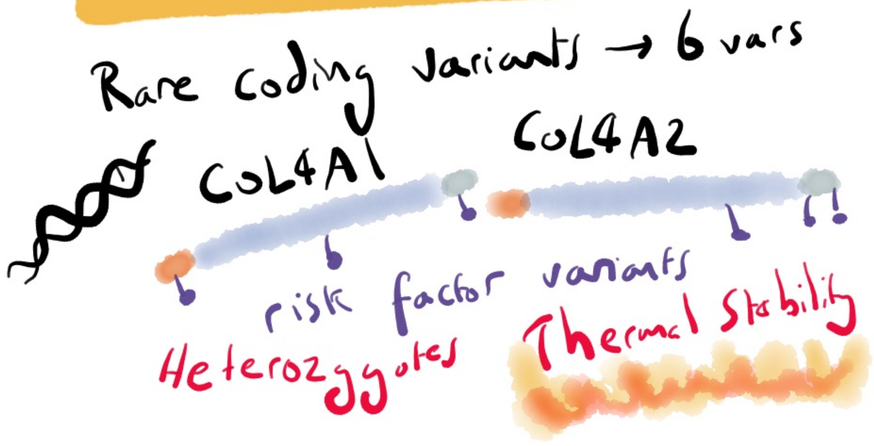
Regulation of ADAMTS-5 expression

Primary cilia mutant chondrocytes

TTBK2

Graham Hamilton

Rare Missense Variants in COL4A1 and COL4A2 as a genetic risk factor in a Sporadic Intra-Cerebral Hemorrhage patient cohort



Alaa Al-Shaer

Sequence-dependent flexibility mapping of Collagen

Triple-helical interruptions - affect collagen flexibility?

Interruptions provide flexibility

Thermally unstable regions

Imaged pN-II collagen

Christine Chew

Kidney Macrophage Heterogeneity and contribution to matrix homeostasis

Kidney Macrophage Heterogeneity

Reporter mice

Implicated in kidney disease

CX3CR1 - marker

Enriched Matrix transcripts in kidney macrophage subsets

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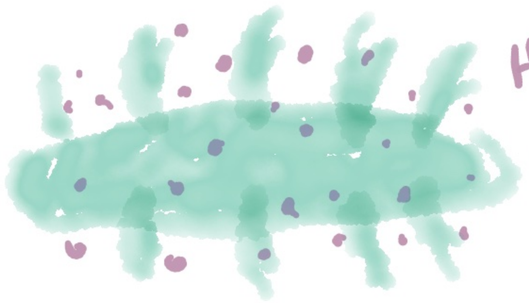
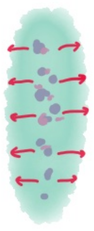
Building an Embryonic Basement Membrane from Scratch



Drosophila Embryo Devo

Hemocytes distribute basement membrane components as they migrate



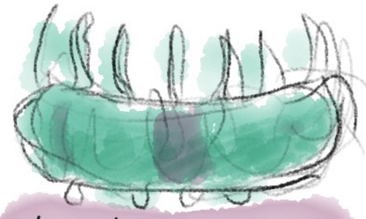
Hemocyte dispersal precedes basement membrane maturation

Protein induction shows logistic growth

Mathematical Model of BM component expression dynamics.

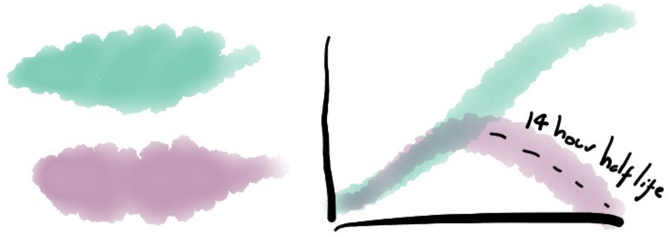
$$dP/dt = S_p M - D_p P$$

Brian Stramer



Local cleavage of ECM affects global VNC condensation

Pulse-chase analysis of Collagen IV turnover in the embryo



Exogenous MMP induction increases ColIV turnover rate

Nidogen loss destabilises ColIV network

Loss of MMP1 decreases the rate of VNC condensation

What is the role of the ECM during VNC condensation?

2 phases

Can ECM polymerization directly produce force?

- Like Shrink Wrap?

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Stably Linking Basement Membranes
Insights from C. elegans

David Sherwood

Cell adhesion systems are crucial for organismal development.

Basement Membrane - Basement Membrane adhesions are poorly understood.

eg Blood-Brain Barrier

163 Human BM genes

75 C.elegans BM genes

B-LINK

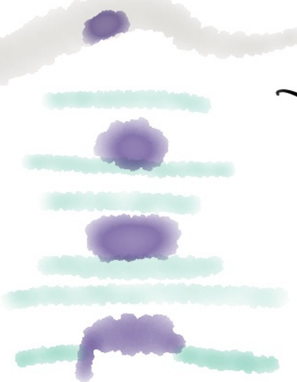
B-LINK helps tissues resist the physical stress of egg-laying

Risk of rupture

C.elegans model system
- CRISPR possible

Transient BM adhesion

C.elegans uterine-vulval connection



UTSE - Seam B-Link connection assembles during mid-to-late L4 developmental stage

Collagen is crucial for B-LINK maintenance

Hemicentin important for B-LINK establishment

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Mechanisms of Leukocyte Penetration of Endothelial Basement Membranes

Endothelial BM

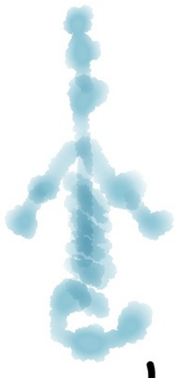
More than just a physical barrier, a checkpoint

Basement membrane is a formidable barrier to cells

laminins, Nidogens, Collagens, Perlecan/Agrin

Molecularly + functionally distinct BMs

Laminin heterogeneity



Integrins α6β1 + αvβ1 required for Laminin 511 recognition

Lydia Sorokin

CD4cre::Itgav$^{-/-}$

T-cells migrate faster into the CNS

Cerebral Blood Vessels

} 2 basement membranes

Endothelial + Parenchymal BMs

Murine Experimental Autoimmune Encephalomyelitis

Extravasation occurs across Postcapillary Venules

Lama4$^{-/-}$ mouse → Induce EAE → - less severe disease

Laminin 511 inhibits Encephalitogenic T cell Migration

Laminin 511 suppresses In Vitro Th17 & Th1 differentiation

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ECM and Basement Membranes in Health & Disease

FLASH TALKS

Anna Gatseva

Identification of Candidate Suppressors of Collagen type IV related disorders in the nematode C.elegans

Basement Membrane
Key Component is Collagen IV

Het Muts in Collagen IV = Pathogenic

Temp sensitive lethality

C.elegans model

Saturation mutagenesis
- look for modifiers
>50,000 worms
2500 modifiers

Ramla Omar

Identifying disease mechanism of vascular Ehlers Danlos Syndrome

Col3A1 mutation - mechanism unknown

Collagen folding issue

Delays in folding

Intracellular Col3 retention

Chemical chaperone treatment for EDS?

Ellen LeMosy

Knockdown of basement membrane associated Tinagl1 disrupts cilia-dependent processes in zebrafish embryogenesis

Tinagl1

Ambiguous function

Zebrafish model knock-down

ciliopathy Syndrome

Grace Wilson

Molecular and skeletal characterisation of mice with a fibrillin-1 mutation; insight into tissue bioavailability of TGFβ in Marfan Syndrome

Skeletal aspects of Marfans.

Mouse model
- aortopathic + skeletal features

Kyphoscoliosis

Growth Plate Histology

Changes in Bioavailability of TGFβ

Sara Jørgensen

Phenotypic changes to vascular smooth muscle cells induced by extracellular matrix modification

ECM maintains vascular cell phenotype

Devo of Atherosclerosis

Phenotypic switch of VSMC driven by ECMs?

VCAM1 expression changes

Peroxynitrate-modified ECM can change VSMC phenotype

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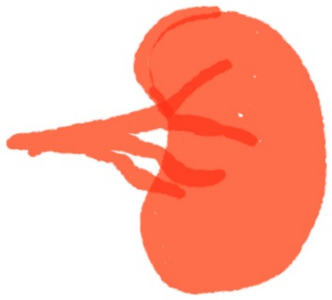
What's the "FUS" about Fibrosis?

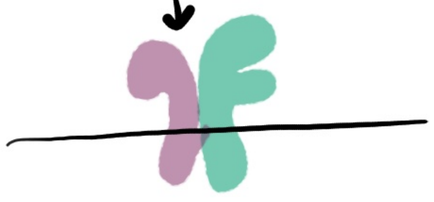


The kidney

Mechanisms controlling extracellular matrix synthesis + degredation

Role of Matrix receptors

ECM



Integrin α1β1 - major binder of collagen binding receptor (IV, I)

- regulates collagen synthesis

Ambra Pozzi

Novel pro-fibrotic

FUS a collagen regulator

Mass Spec - reveals target for collagen transcription

RNA/DNA binding protein FUS/TLS

- if FUS localises to Mitochondria causes cell stress - step in ALS

ALS = ↑ collagen in the skin

Itga1KO cells

- FUS form ↑ complex with EGFR

EGFR-mediated FUS phosphorylation induces FUS nuclear transportation

FUS-NLS derived peptide prevents collagen production

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LaNt alpha31: the "Missing" Laminin-Superfamily Member

LaNtα31

- adding to a key family

laminin α chains

} LaNts

α netrin1

- created through alternative splicing?

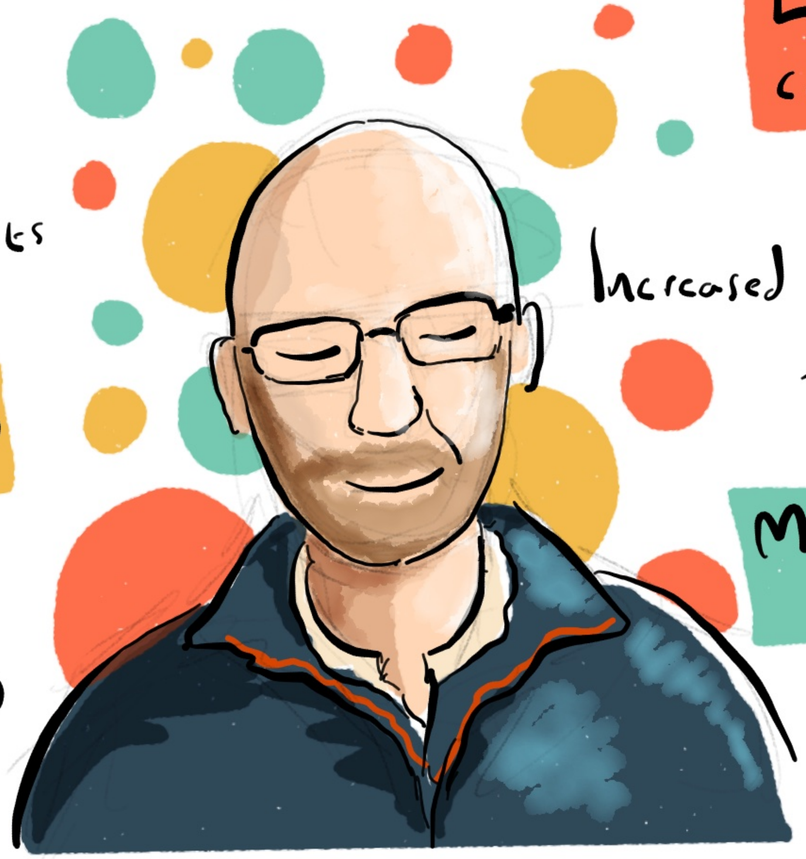
LaNts

Where + when are they expressed?

Widely expressed across tissues

Different tissues express different Laminins

Kevin Hamill



LaNtα31 upregulated during corneal wound re-epithelisation

Increased LaNtα31 expression in epithelial cells

- Increased 2D spread area

Mature hemidesmosomes form on LaNtα31

Increased processing of laminin α3

LM clustering

MMP activity

} causality?

Inducible LaNtα31 overexpressing inducible mouse line

Subtle changes in the skin

Kidney - Interstitial bleeding

Lung - thickening of alveolar membrane

Laminin genes are regulating themselves!

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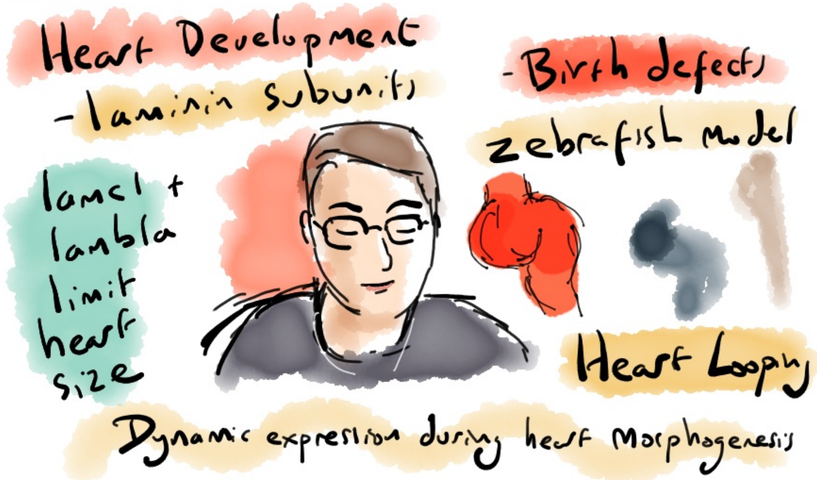
Matrix Secretion, Assembly & Turnover

Mychel Morais

Investigating basement membrane assembly and matrix remodelling during Kidney development

Chris Derrick

Laminins regulate cardiac growth through restricting Second heart field addition



Marion Baraban

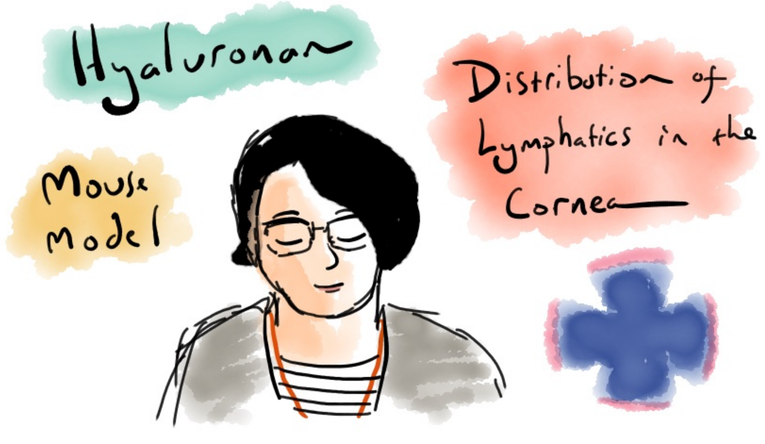
Role of the basement membrane and the basal cells in the regulation of skin integrity in zebrafish embryo

Ben Johnson

A CRISPR/Cas9 in vitro human induced pluripotent stem cell (hiPSC) model to investigate the role of perlecan in cardiovascular fibrosis

MingXia Sun

Hyaluronan derived from the limbus is a key regulator of Corneal lymphangiogenesis



Circadian Clock Control of Collagen Homeostasis

New Tools and Approaches

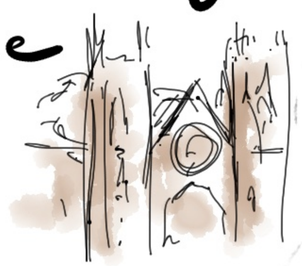
Karl Kadler

How do we make and maintain extracellular matrix?

We are mostly extracellular matrix!

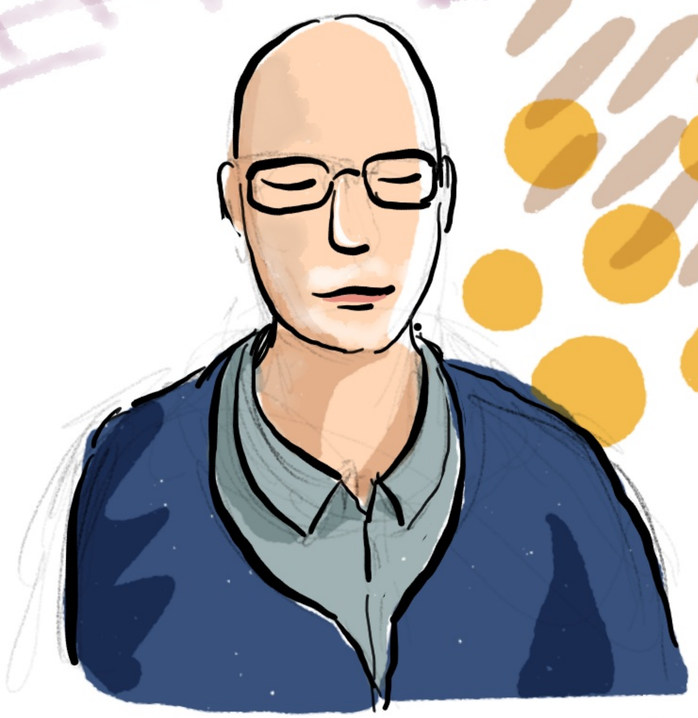
Collagen degradation & Ageing

Buildings are made once then degrade
- require maintenance



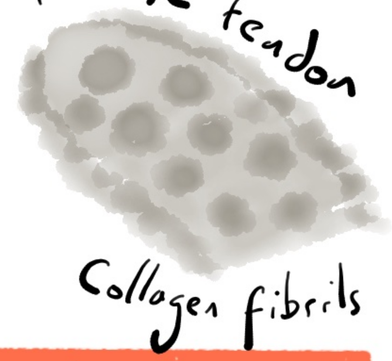
Some cells continue to make collagen

Tendon is a peripheral circadian clock tissue

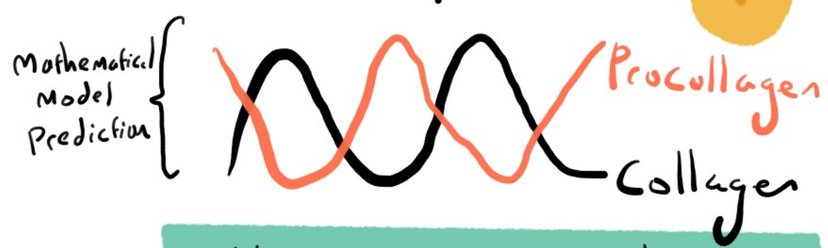


Quantifying Matrix Synthesis

Nanoluciferase



Fibril diameters vary with time of day
- D1 change over 24 hour period



10% of proteome in tendon is 24 hour rhythmic

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Understanding the Role of Extracellular Matrix Glycosaminoglycans in Facilitating Chemokine Mediated Immune Cell Recruitment and Inflammatory Disease

Douglas Dyer

Transendothelial leukocyte Migration

How cells get from the blood to tissues

Chemokines - central to inflammation
- Linked to arthritis, asthma, metastasis...

Limited success of therapeutic interventions

Need to develop our knowledge

Why have interventions failed?

The ECM → Glycocalyx - adhesion-resistant barrier (0.2-2 μm)

Chemokine Redundancy? Perhaps Not

Chemokine Expression Atlas

Matrix interaction differentiates between Monocyte chemokines

GAG interactions critical for cell migration

role of CXCL4

Why are GAG interactions critical?

Extracellular Matrix function may explain major gaps in our understanding of chemokine biology

CXCL4 binds to glycosaminoglycan sugars with high affinity due to oligomerisation

HS GAGs directly facilitate Chemokine function

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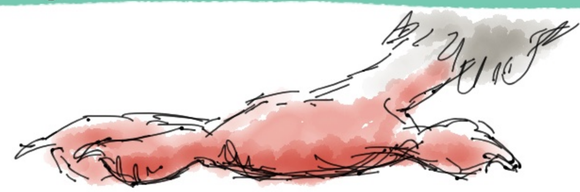
From Target Identification to Symptom-Relief Therapies for a Basement Membrane Disease

Alex Nyström



Collagen VII

- recessive dystrophic epidermolysis bullosa

Progressive fibrosis disorder

wounds ⇒ scarring ⇒ skin cancer

Disease Progression & Therapeutic Targets

Starts with Collagen VII deficiency

Early Intervention Most Effective

Losartan → TGFβ1 ← Decorin

TGFβ1 → pSMAD2/3

JAK1/STAT3 → Ruxolitinib

RhoA/ROCK

Once Daily Administration of low dose of Ang-(1-7) extends the survival of RDEB Mice

Dermal fibrosis Mouse model

RDEB Mouse

Mass-spec analysis of Proteome

- Fibrin clot formation changes

↑ Inflammatory Macrophages in all stages

- Alterations in ECM

Activation of adaptive immunity (type 1)

Kininogen-1

- increased during progression to fibrosis

Ang-(1-7) protractedly limits fibrosis-promoting capacities of inflammatory cells and fibroblasts

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ECM and Basement Membrane in Health & Disease

Paul Potter

A Novel Model of Nephrotic Syndrome Results from a Point Mutation in Lama5 and is Modified by Genetic Background

Nephrotic Syndrome (chronic)
mice with chronic renal failure

E884G mut in LAMA5

Phenotype modified by genetic background

25 weeks

Histology differences

Eileen Gentleman

ILC1-Derived TGFβ1 Drives Epithelial and Matrix Remodelling in Human iPSC-Derived Intestinal Organoids

Innate Lymphoid Cells

Repair → Pathology

- rare immune cell in the gut

Type 1
what does it do?

ILC1
- accumulates in Crohn's

Organoid Model

Drives epithelial & Matrix Remodelling

Synthetic Hydrogel

Keerthi Harikrishnan

Fibulin 1 is Critical for Anterior, Left Coronary, Non Coronary Leaflet Formation During Pulmonary and Aortic Valve Morphogenesis

Semilunar valve formation

Fibulin-1
- expressed in developing heart

Left + non Coronary affected

Mouse Model

Differences in Apoptosis

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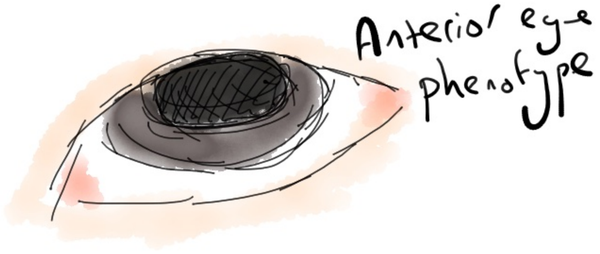
Towards a Comprehensive View of the Physiology and Pathology of Collagen 18

Taina Pihlajaniemi

Mutations in ColXVIII cause Knoblach syndrome



Ubiquitous in Basement Membranes

Occurs in 3 Isoforms

Promoter specific KO mice

Brown Adipose tissue

role of Col18 in thermogenesis

Viable KO mice

- reduced adiposity + enlarged liver

Lipodystrophy + perturbed lipid homeostasis

Altered serum lipid profiles

Altered Glucose Metabolism

Non-shivering Thermogenesis

- BAT activation

Also regulates nephron progenitor cell fate

COL18 Regulates Glucose & Lipid Metabolis

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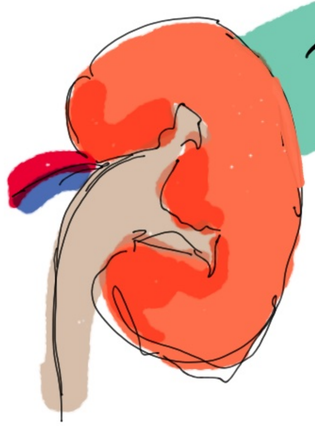
Matrix, Cells, and Kidney Glomerular Filtration

Jeffrey Miner

The Kidney



The Glomerulus

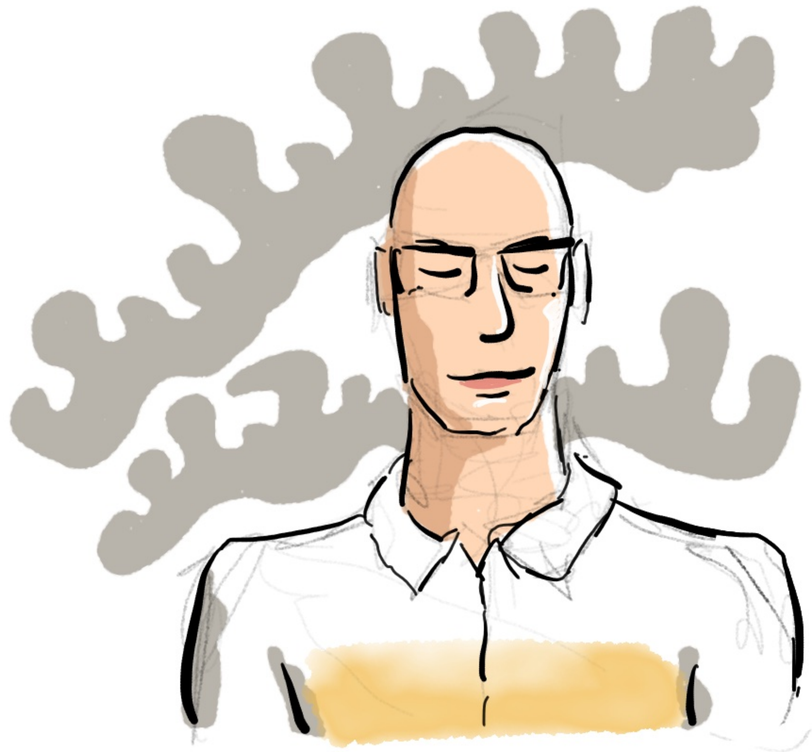
Glomerular Filtration Barrier

Dual Devo Origin of GBM

Epithelium (podocyte) + Endothelium

Hemicentin-1

→ No phenotype in KO mice



Genetic Manipulation - Synaptopodin

- KO mouse
- No overt kidney defects
- With Adriamycin Nephropathy have worse outcomes

Alport Syndrome = Defective GBM composition, structure + stiffness increase strain on podocytes by capillary blood pressure

Hemicentin 1 in Glomeruli

Mutations in Major Glomerular Basement Membrane proteins cause disease

Alport Syndrome

- hereditary glomerular disease

Split, Thickened 'Basket Weave' GBM

Alport Glomeruli are less stiff by Atomic Force Microscopy

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