Regulation of Joint Development by Mechanical Factors

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• Variation in limb proportions
• Joint shape and position?
• Huge impact for locomotion
• Emerge during development
• Role of mechanical input?
Cartilage growth:
• Proliferation
• Maturation
• Hypertrophy

Move very early – day 5
Hamburger, Balaban, 1963
Joint 'cavitation'

Skeletal elements distinct from the outset

Joint morphogenesis

Patterning normal without movement

How does movement contribute to joint formation?

Does movement contribute to limb proportionality?

Mechanism of cavitation

in ovo paralysis

Fell and Canti, 1934
Movement drives joint-formation and limits default chondrocyte fate

Control

Immobilised

sGAG

UGDH

CD44

FGF2

Stages 36-39
TT joints

Stages 36-37

FGF2

Dowthwaite et al., Matrix Biol, 2003
Bastow et al. JBC 2005
Kavanagh et al. Dev Dyn. 2006
Movement activates ERK selectively at forming joint surfaces

**Phospho-ERK** Propidium iodide

*Bastow et al. JBC 2005*

**p38 kinase** Lewthwaite et al., JBC. 2006

**COX-2** Wheeler et al., under review

**ERK function in HA synthesis/binding**

**Coat size**

<table>
<thead>
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<th>Control</th>
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<tbody>
<tr>
<td>0.6</td>
<td>0.8</td>
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**Coat size**

<table>
<thead>
<tr>
<th>Control</th>
<th>DN-MEK1</th>
<th>CA-MEK1</th>
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<tbody>
<tr>
<td>1.0</td>
<td>0.4</td>
<td>1.6</td>
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* denotes statistical significance.
Muscle Contraction Is Necessary to Maintain Joint Progenitor Cell Fate

- sploch delayed mutant (Spd): Pax3 mutation defect in muscle progenitor cell migration
- Myf5/MyoD KO - no myoblast formation
- Spd: Reduced proliferation at forming joints

Gdf5-Cre mice crossed with R26R-lacZ reporter mice

- Joint progenitors lose normal behaviour & differentiate into chondrocytes

Kahn et al., (2009)
Dev Cell. 16:734-43
Mechanosensitivity of the interzone is acquired

Early
d4-d7

Control

Gli3

Wnt4

Col2a1

Gdf5

NO CHANGE

Flaccid

Late
d7-d10

Control

Sox9

IZ ➔ Cartilage

Flaccid

Joint formation but not patterning

Yulia Shwartz & Elazar Zelzer (collaboration)
Plasticity of interzone cells is key fate-determining step

Too many differentiate into chondrocytes (lost from interzone) then joints fuse

Too many remain in interzone pool, then skeletal elements won’t grow sufficiently

Movement-induced mechanical stimulus converge upon ‘pro-inflammatory’ signalling?

Mechanisms responsive to movement that define these boundaries are crucial

Bastow et al., JBC, 2005 (ERK)
Lewthwaite et al., JBC. 2006 (p38^MAPK)
Wheeler et al., submitted (COX-2)
Are changes in chondrocyte behaviour in OA mechanically-guided?
NFκB signalling dominates gene expression in cartilage predisposition, onset and progression in OA-prone mice

**OA predisposition – Str/ort v. CBA 8wks**

139 up- and 0 down-regulated genes

Collagen VIII and XII  
Tenascin C  
MMP19  
Htra1  
TIMP1

IPA:  
- Genetic  
- Connective tissue disorders  
- Cell death

Centred on **NFκB signalling**  

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Davidson et al. 2013 A&R  
Javaheri et al., 2015

Stable synthetic sulforaphane improves bone architecture & gait in Str/ort  

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Evgen
Mechanical input required for joint cavitation & normal skeletal growth

• Osborne et al., JMNI, 2002
• Lamb et al, IJEP, 2003
• Limb proportions?

Andrea Pollard
Pollard et al, 2014 Journal of Anatomy

Chick - Ostrich - Crocs

Gustavo Hernandez, Mexico
- Define critical time-points
- Determine if impact on skeletal growth is due to proximo-distal sequence of development
- Identify affected phases of endochondral ossification

- Embryo movement starts early in development
  - day 5 in chicken, week 7 in humans
  - evidence that this serves a critical role
Unpublished data
Unpublished data
Unpublished data
Movement differentially drives skeletal element growth, after a certain developmental time-point, to modify limb proportionality.

Is the differential growth achieved in this final hypertrophy phase partly dependent on movement?
Unpublished data
Unpublished data
Type IIB collagen

Aggrecan-rich HA-enriched

Has2-/-
Sox9-/-

Type IIA collagen

HA-deplete
N-Cad, NCAM-1
tenascin-C and Sox9

DDR2
Ugdh

Type IIB collagen

Aggrecan-rich HA-enriched

Proliferation
Differentiation

Condensation

Wnt-7a, Shh
FGF2/4/8, TGFβ
Hox, Pax, bHLH

Apoptosis

Anp32a

Chondrocyte
Terminal Differentiation
(MMP13/Col10
VEGF/RUNX2)

Is OA-related transition in chondrocyte behaviour mechanically-guided?
Unpublished data
Increased bone bridging across growth plate in pre-OA STR/Ort mice.

40 wks

STR/Ort

CBA

40+ wks
Valgus 1.7 cm longer

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Andrea Pollard
Mark Hopkinson
Gustavo Hernandez
Blandine Poulet

Gary Dowthwaite
Anne Osborne
Emma Kavanagh
Katherine Lamb
Edward Bastow
Jo Lewthwaite
Ben Wheeler
Roberto de Souza

Elazar Zelzer (Israel)
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