Skeleton

Flexibility

Protection of vital organs

Strength
Skeletal defects

Developmental defects

Degenerative diseases

Fibrous dysplasia (fibrous tissue develops in place of normal bones), Cleidocranial dysostosis (defect in cranial bone)
CHEMICAL COMPOSITION OF BONE

- **MINERAL**
- **LIPID**
- **NON-COLLAGENOUS PROTEIN**

**BONE**

- **ORGANIC**
  - 35%

- **INORGANIC**
  - 65%

**RESISTS EFFECT OF PULLING-TENSION**

**MATRIX**

**CELLS**

**MAINLY HYDROXYAPATITE**

**RESISTS BENDING AND COMPRESSION**
Bone cells

- **Osteoblasts**: osteoblasts are the major cellular component of bone. Bone matrix is mineralized by the osteoblasts. Osteoblasts buried in the matrix are called **osteocytes**.
- **Osteoclasts**: Osteoclasts break down bone tissue.
- **Bone marrow**: Bone marrow is the tissue of the interior of the bone. It contains blood vessels that supply oxygen and nutrients for bone formation.
Diseases of bone

• Osteoporosis
• Rickets
• Paget’s disease
• Osteogenesis imperfecta

Diseases of Bone Joint: A joint disorder is termed arthropathy

• Osteoarthritis
• Rheumatoid arthritis
• Gouty arthritis
• Temporal mandibular Joint syndrome
Joints

- Joints hold the skeleton together and support movement.
- There are two ways to categorize joints.
  1. The first is by *joint function*, also referred to as *range of motion*.
  2. The second way to categorize joints is by the material that holds the bones of the joints together; that is an organization of *joints by structure*.
Category of joints according to their function

• Synarthrosis (range of joint motion: no movement): Skull Sutures
• Amphiarthrosis (range of joint motion: little Movement): distal joint between the tibia and the fibula and the pubic symphysis
• Diarthrosis (range of joint motion full movement): Elbow, shoulder, ankle
Category of joints according to their structure

• **Fibrous Joints.** Between the articulations of **fibrous joints** is thick connective tissue, which is why most (but not all) fibrous joints are immovable (synarthroses). Eg: **Sutures** are nonmoving joints that connect bones of the skull.

• **Cartilaginous Joints.** Joints that unite bones with cartilage are called **cartilaginous joints**. Eg: hip bones, connected by the pubic symphysis.

• **Synovial Joints.** These are characterized by the presence of an articular capsule between the two joined bones. Bone surfaces at synovial joints are protected by a coating of articular cartilage.
Zebrafish: A model to study bone joint formation
Fin regeneration proceeds rapidly

Amputated fin  After regeneration

Skeletal precursor cells
Proliferative cells
Amputation plane

Longitudinal section

M Kathryn Iovine, 2007
Advantages to examining growth mechanisms in the zebrafish fin

• The fin grows throughout the lifetime of the fish.
• When amputated, the fin grows back (regeneration) within short span of time.
• Fin has multiple fin-rays flanked by joints.
• It is easy to distinguish between the newer joints from the older joints.
• Zebrafish are easy to manipulate genetically.
Fin length mutants reveals skeletal defects

Bone growth mutants

Short fin mutant

Another long fin mutant

Misu et al., 2016
sof phenotype

Short fin length  
Decreased cx43 mRNA  
Short segment length

Another long fin phenotype (alf)

Longer fin length  
Increased cx43 mRNA  
joint failure: longer segments
Identification of a gene responsible for normal bone development- Connexin43

Mutations in the *connexin43* cause the *short fin* phenotype.

The *short fin* mutant exhibits less *connexin43* mRNA, which leads to less growth and shorter bony segments.
Cx43 is the main component of Gap Junctions.

(a) Direct communication through gap junctions

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Gap junctions facilitate the exchange of small molecules between cells

- Ca²⁺
- Electrical Currents
- Second Messengers (IP3)
- Proteins
- Nucleic Acids

7 nm 1.5 nm 17 nm
Loss of Gap Junction Proteins Leads to Disease

- cx43-/- leads to heart malformation
- cx46-/- leads to cataracts
- cx37-/-;cx40-/- leads to defects in vasculature
Gap junctions are:

- Channels that reside in the plasma membranes of adjacent cells.
- Major sites of direct cell-cell communication of small molecules.
- Required for cell and tissue function
  - Onset of labor
  - Conduct neuronal signal through electrical synapses
  - Heart Beat
Cx43 function is conserved and is required for skeletal morphogenesis in vertebrates.

- Defective Cx43 function causes skeletal defects in human, mouse and chick.

Oculodentodigital dysplasia (ODDD)

Musa et al, 2009
Cx43 mutation causes sof and alf phenotype in zebrafish

sof phenotype

Short fin length
Decreased Cx43 mRNA
Short segment length

another long fin phenotype (alf)

Longer fin length
Increased Cx43 mRNA
stochastic joint failure: longer segments
All methods that reduce Cx43 activity (like in the sof mutant) causes reduced fin growth, reduced cell proliferation, and reduced segment length.

An increase in Cx43 (like in the alf mutant) leads to increased fin growth, increased cell proliferation, and increased segment length.
Model for Joint Formation

Skeletal patterning

Skeletal growth

cx43

What happens after Cx43? What is controlling Cx43?
Joint initiation is similar in fin rays and long bone

Fibrous joints  Synovial joints

The first sign of joint formation is the appearance of interzone.

Sims et al., 2009
Interzone is established at 87 hours post amputation (Hpa)

- **72 Hpa**: Pre-interzone/Preinitiation
- **87 Hpa**: Interzone/initiation: Single layer of elongated cells
- **96 Hpa**: Post Interzone/Post initiation: Spherical shaped cells forming two layers
evx1 is required for joint initiation

- The evx1 gene is expressed in a single band of cells at the distal end of fin rays as they produce joints

\[ cx43 \rightarrow evx1 \rightarrow \text{skeletal patterning} \]
Our Research Questions

1. What is controlling Cx43?
2. How is Cx43 suppressing evx1? What is the mechanism by which evx1 is increased during joint initiation? \textit{cx43} \rightarrow \textit{evx1}
3. How many pathways are there for joint formation?
KD of *smp* results in shorter segments in fins

Smp expression is in the mesenchyme (m), underlying the epithelial cap (ec) and basal epithelium.

Kizil et al., 2009
Do $cx43$ and $smp$ act together?

Test 1: Does $smp$ expression depend on $sof$?

- Wild type
- short fin ($sof$)
- another long fin ($alf$)

Cx43
down

Premature joints

Smp?

Cx43
up

Joint failure
$smp$ expression requires $cx43$

- In-situ hybridization for $simplet$ in $Wt$, $sof$ and $alf$ in 3 DPA fins

$alf$ Increased in $alf$  

$Wt$ Reduced in $sof$

- $smp$ expression follows $cx43$ expression pattern
Do \textit{cx43} and \textit{smp} function together?

Test2: test for synergy

\textit{cx43} \rightarrow \textit{smp} \rightarrow \text{evx1} \rightarrow \text{skeletal patterning}

Synergy concept

Cx43 is reduced \rightarrow \text{short segment}

Smp is reduced \rightarrow \text{short segment}

When both \textit{cx43} and \textit{smp} is reduced \rightarrow \text{much shorter segment}
Transient gene knockdown using morpholinos

Morpholinos (MO) bind nascent RNA and block protein TRANSLATION via steric hindrance.
Morpholino mediated knockdown during fin regeneration

1) Amputate the fin

2) At 72HPA
   - Place fish in dish
   - Orient needle
   - Inject above each ray
   - Electroporate
   - Do not touch fin tissue

3) 

4) Harvest the 4dpe fins for joint assessment or 1dpa fins for gene expression and H3P assessment. Measurement is taken on the third fin ray.
Smp regulates skeletal growth

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**Legend:**
- **Uninjected side**
- **Injected side**

**Graph:**
- **Smp MO**
  - Uninjected side
  - Injected side
- **STD MO**
  - Uninjected side
  - Injected side

**Axes:**
- **X-axis:** Regenerate length (µm)
- **Y-axis:**
  - 0
  - 200
  - 400
  - 600
  - 800
  - 1000
  - 1200
  - 1400
  - 1600

**Notes:**
- * indicates a statistically significant difference.
Smp regulates joint patterning

![Images showing segment length comparison between Std MO injected and uninjected sides, and Smp MO injected and uninjected sides.](image)
Test if $cx43$ and $smp$ lie in the same pathway

- **Inject Smp Mo**: Shorter than the $sof$ segments
- **Inject Std Mo**: Shorter than Wt. No difference

### Subthreshold dose in Wt is 0.5mM

When this 0.5mM dose is injected in $sof^{+/-}$, the segment length should be shorter than the wt.

- Injected side
- Uninjected side
Cx43 and Smp function in the same pathway to regulate joint formation

![Graph showing segment length measurements in WT, sof/+.](image)

Fig 2: Segment length measurements in WT, sof/+.
Smp regulates evx1 expression during joint morphogenesis

Cx43 → Smp → evx1 → Skeletal patterning

smp morpholino injected fin std morpholino injected fin

Smp regulates evx1 expression during joint morphogenesis.
Conclusion from my research work

- *smp* knock-down phenotypes are similar to *cx43* knock-down phenotypes i.e., reduction in fin regenerate length and segment length.
- *cx43* and *smp* appear to regulate joint formation by functioning in a common molecular pathway.

Cx43 $\rightarrow$ Smp$\rightarrow$ evx1 $\rightarrow$ Skeletal patterning
Future Directions

- The most important question in this study is what is regulating cx43 expression during joint patterning.
- Future studies are aimed at revealing the mechanisms by which evx1 is regulated by smp during joint initiation.
Acknowledgements

Iovine lab - Lehigh University
Dr. Kathy Iovine

NIH for funding

Annie Sanchez
Cassandra Field
Rebecca Bowman
Dominick
Kerolos Aziz
Layna
All the past members