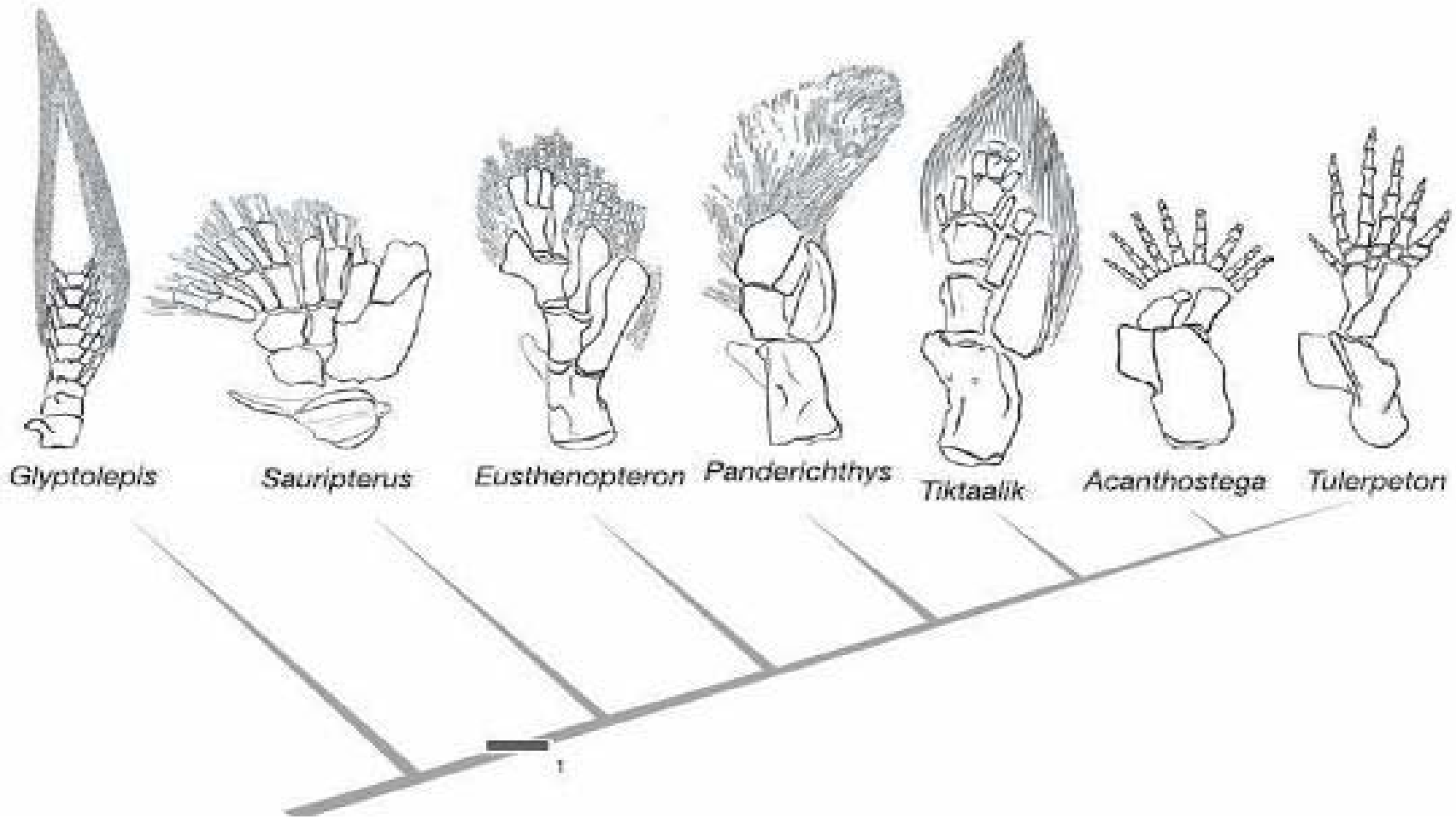


# Chapter 10: Postnatal Growth of Fins and Limbs through Endochondral ossification

Cornelia E. Farnum

Review by Susan Lujan

# Limb diversity



# BBB

## Basics of Bone Building

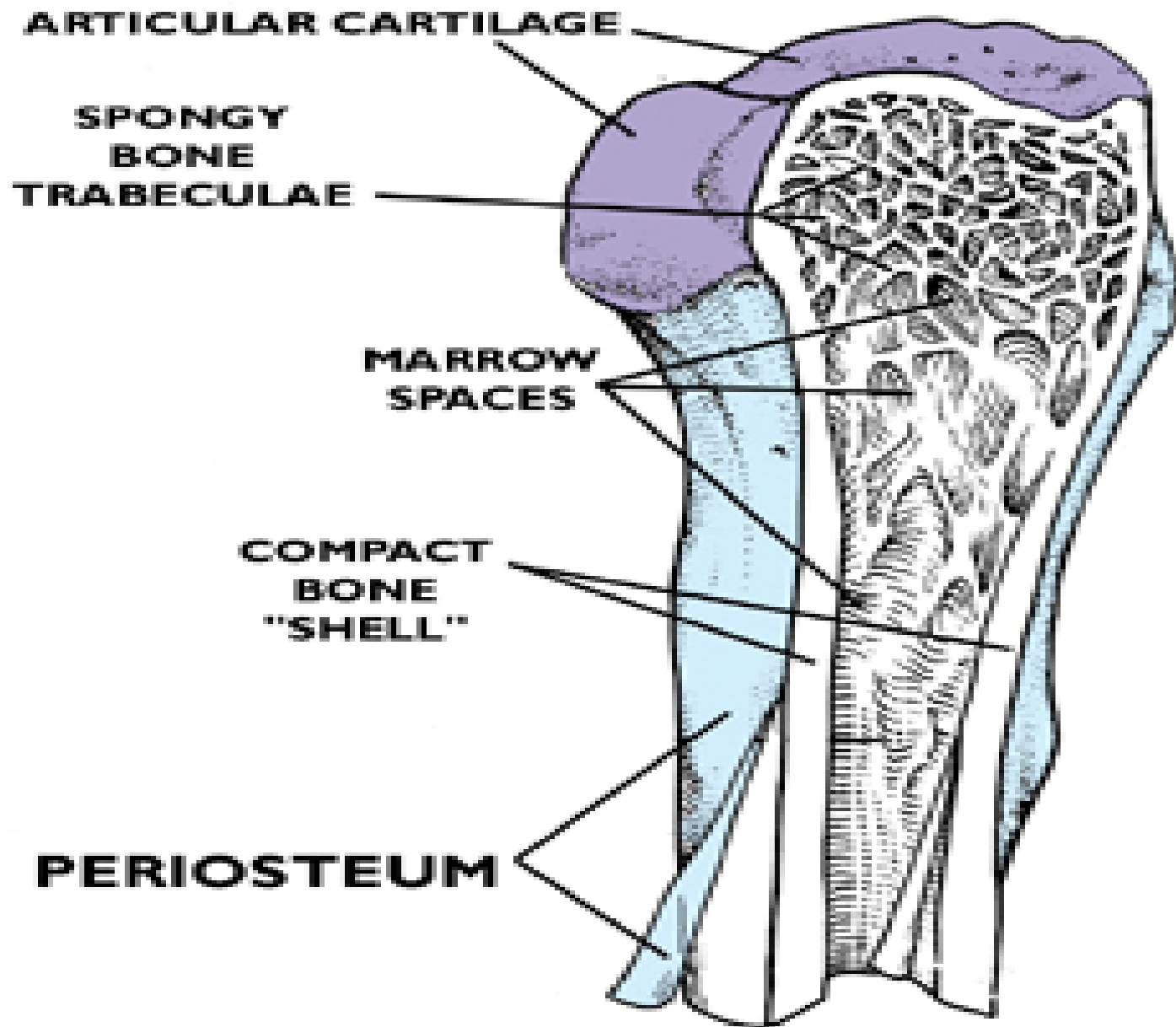
- Bone
  - CT
    - with cells, ordered fibers, mineralized matrix
    - Relatively light weight, strong, resilient
    - Arises by replacement of pre-existing tissue
      - Endochondral ossification (long bones)
      - Intramembranous ossification (skull)
  - Dynamic
    - Calcium, phosphorus reservoir
    - Continual modification/remodeling
    - Physiological function (marrow/hemopoiesis)

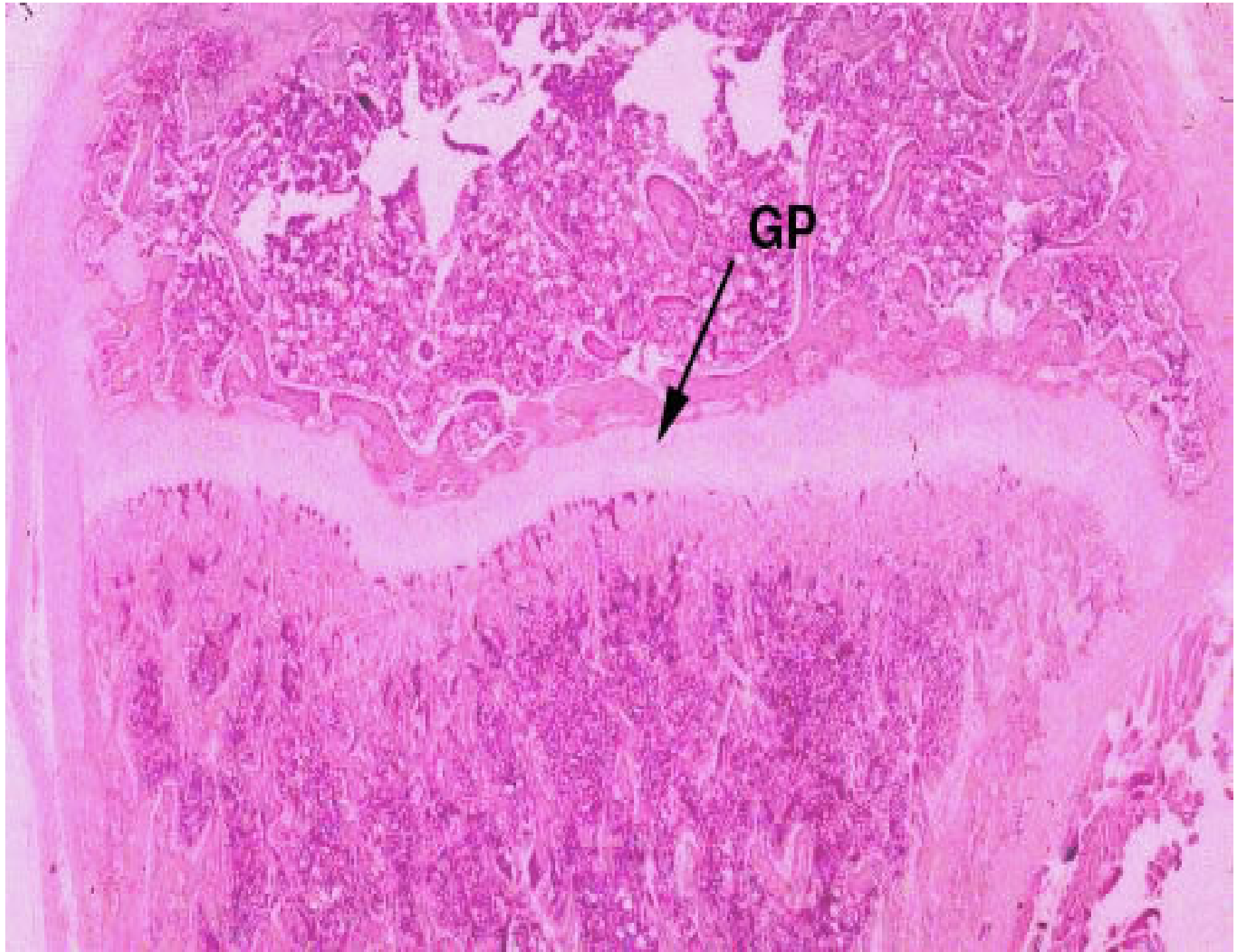
# Long bone

- Endochondral ossification (elongation) as well as intramembranous ossification (surface—maintain shape, thickness, formation of protuberances, condyles, O&I)
- Covered with sheath of compact bone
- Ends contain trabecular (spongy) bone
- Articular ends capped with cartilage
- Diaphysis
  - Covered with periosteum (perichondrium)
    - Contains osteogenic cell layer
- Epiphyses (one epiphysis each end)
  - Growth plate (cartilage)
    - Allows for elongation of bone, before ossification
    - Closure (hormonal) stops growth (distal first)

# Endochondral ossification

- Embryonic skeleton is a hyaline cartilage model
- Primary center of ossification established at center of template
- Blood vessels invade previous avascular cartilage, carrying progenitor bone-forming cells
- At time of birth, most cartilage replaced by bone
- Bones elongate by means of secondary centers of ossification which develop at proximal/distal ends of long bones
- Growth can occur because the cartilage present in epiphyseal growth plate has not yet been replaced
  - Growth plates are between two centers of ossification, formation of bone proceeds toward the plate from both directions, but the growth of cartilage is faster on one side (allows for elongation)







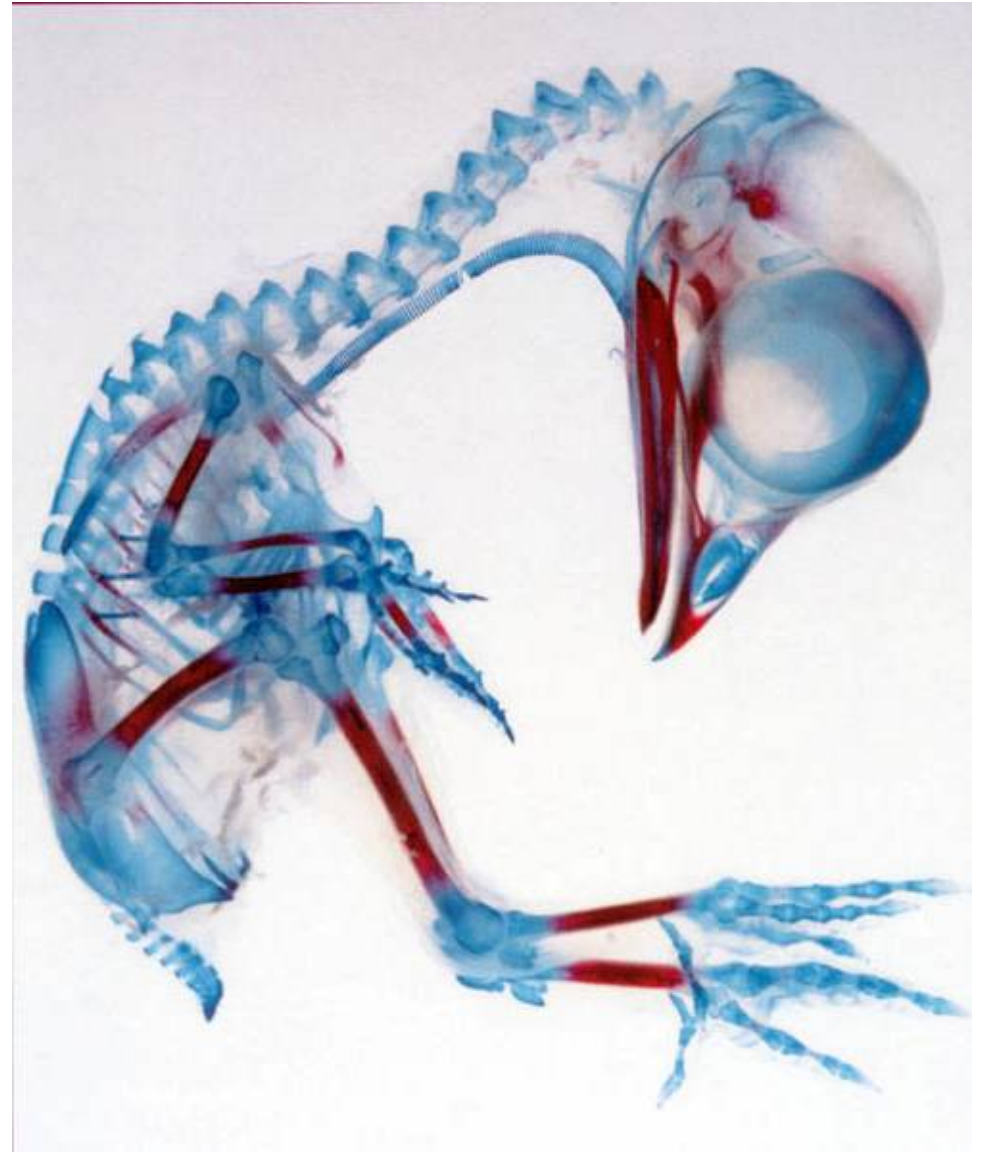
# Growth of cartilage/Replacement by Bone (X2)

- Zone of resting chondrocytes
- Zone of proliferating chondrocytes
- Zone of hypertrophic chondrocytes
- Zone of calcification
- Invasion of blood vessels and osteogenic cells
  - Osteoblasts secrete osteoid on matrix previously laid down by chondrocytes (scaffold)
  - Primary bone (woven, random fiber orientation)
- Primary bone removed by succeeding waves of osteoclasts; then osteoblasts will lay down secondary bone (ordered fibers) and matrix, mineralization occurs.

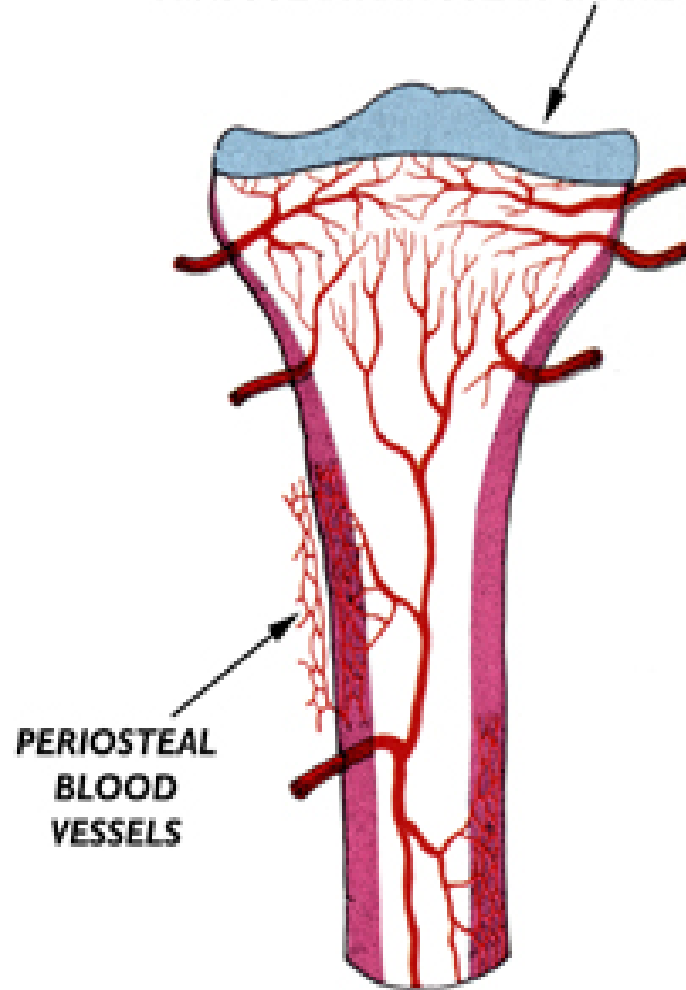
# Cool Chick

- Skeleton of embryonic chick
- Alizarin Red (hardened bone)
- Alcian Blue (remaining cartilage)
- Endochondral ossification proceeds from center of long bone toward the ends. Here, proximal and distal ends of femur, humeri, and radii, have not established secondary centers of ossification but shaft of bones is well under way.

This image, and preceding review of bone formation from Dr. Thomas Caceci



**AVASCULAR ARTICULAR CARTILAGE**



# Recap of Chapter 7



# Limb Chondrogenesis

- Conserved basic skeletal structure of tetrapod
  - Proximal stylopod (humerus)
  - Medial zeugopod (radius/ulna)
  - Distal autopod (wrist, fingers)
  - Formed in same sequence
- Limb skeleton formation via EO
  - Cartilage template size and shape due to precursor cells from mesenchyme: differentiate to chondrocytes and enter cascade, adopt different shapes and alter gene expression (proliferate, secrete matrix, hypertrophy, matrix becomes calcified, then undergo apoptosis)
  - Peripheral cells become perichondrium; later mature into periosteum
  - Inner cell layer of periosteum adopt osteoblast fate, forms the bone collar
- Region of terminal hypertrophic chondrocytes within calcified matrix are invaded by blood vessels, and osteoblast/osteoclast precursor cells.
- Growth plates form at ends of long bones (separate distal, cartilage epiphyses from medial bone diaphysis)
  - Plates made up of chondrocytes; continual cascade and replacement of dead cells by bone

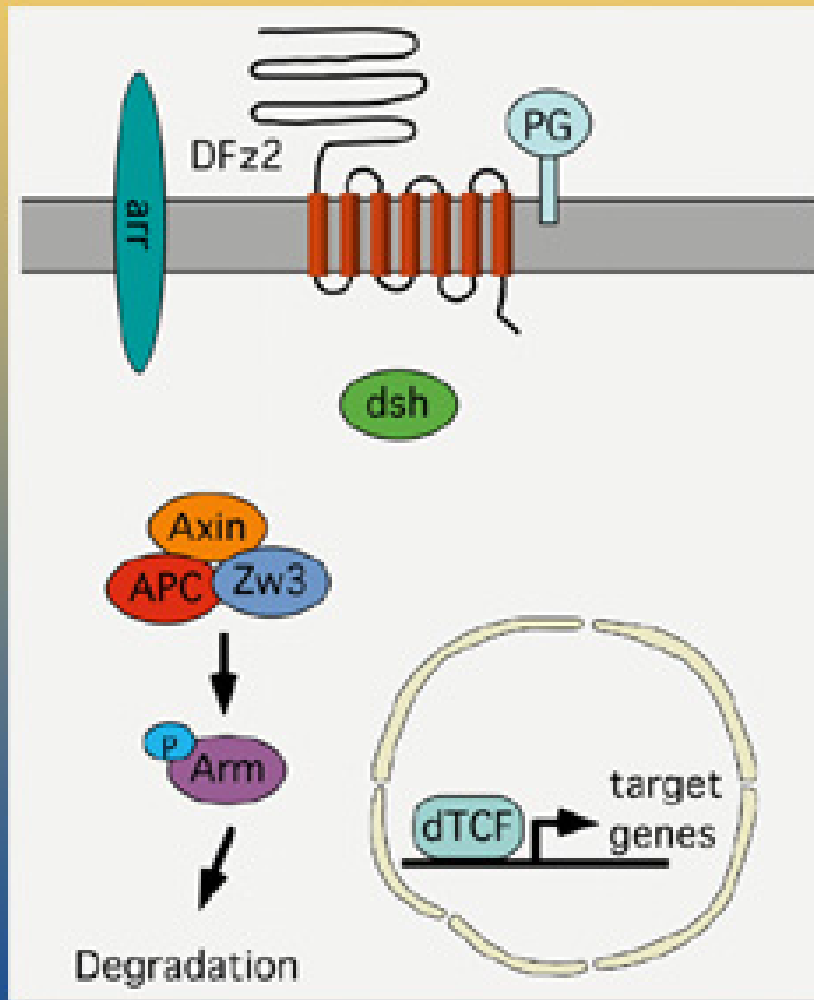
# Control of Chondrogenesis

- FGF
  - negatively regulates proliferation of chondrocytes thru JAK-STAT<sub>1</sub> (upregulation of a cell cycle inhibitor)
- BMP/BMPR (Ia, Ib, II)
  - Acts at several steps:
    - Stimulate prechondrogenic condensation
    - Stimulate differentiation of progenitor cells into chondrocytes
    - Negative regulation of hypertrophic differentiation
- PTHLH/IHH
  - Balance proliferating/hypertrophic chondrocytes
  - IHH synthesized and secreted by chondrocytes in transition zone P/H; control number of chondrocytes undergoing differentiation
  - Also regulates levels of PTHLH (acts to delay differentiation)
- WNT
  - Acts at several stages controlling differentiation (5a inhibits proliferation, 5b promotes)
- SOX
  - Family of Transcription Factors, required for condensation, differentiation
- HOX
  - 9, 10 stylopod
  - 11 zeugopod
  - 13 autopod
  - Regulate longitudinal growth of elements

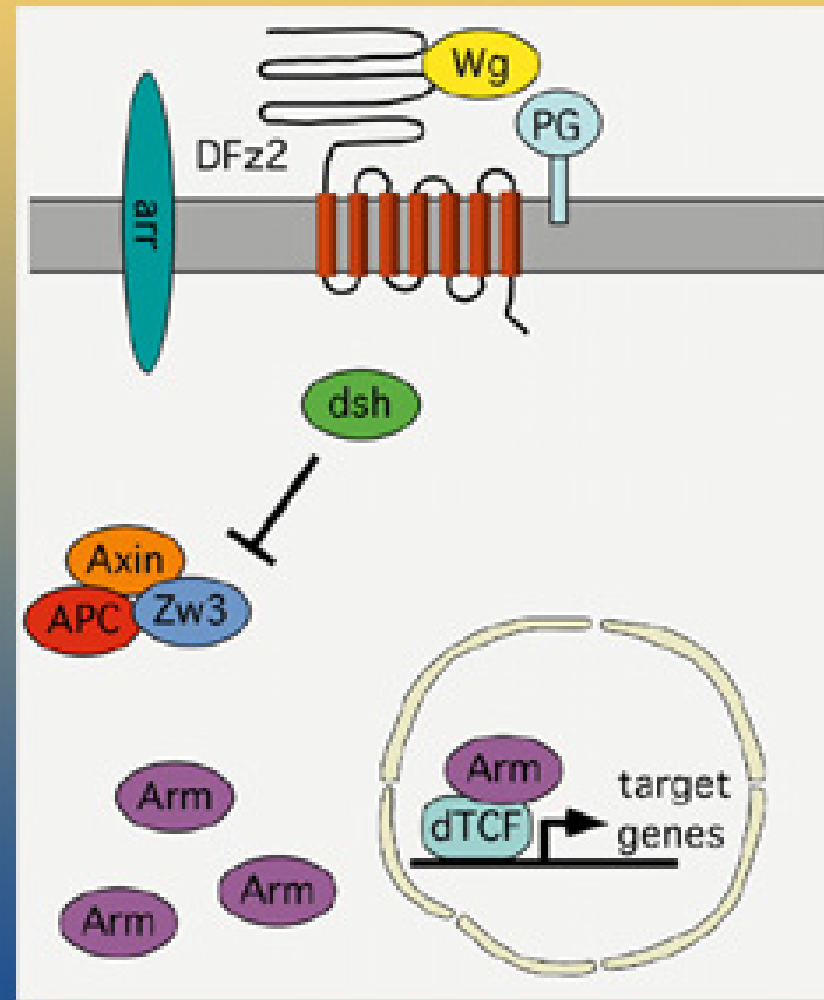
# Limb Osteogenesis

- Osteoblasts/clasts enter cartilage via blood vessels entering calcified matrix (chondrocytes now dead)
- Osteoblasts replace existing ECM with bone ECM
  - Mesenchymal cells, differentiate into either chondrocytes or osteoblasts (as well as fibroblasts)
  - Eventually encased in lacunae as osteocyte (maintenance)
  - Processes and canaliculi (diffusion prevented by hardened matrix) allow for communication
- Osteoclasts
  - From monocytes
  - Primary function in bone resorption/remodeling/contouring (necessary as new bone formed)
  - Attach to matrix, undergo shape change (ruffled) and enzymes such as TRAP and CATK can degrade minerals and collagen

# The WNT/Wg signaling pathway



Inactive

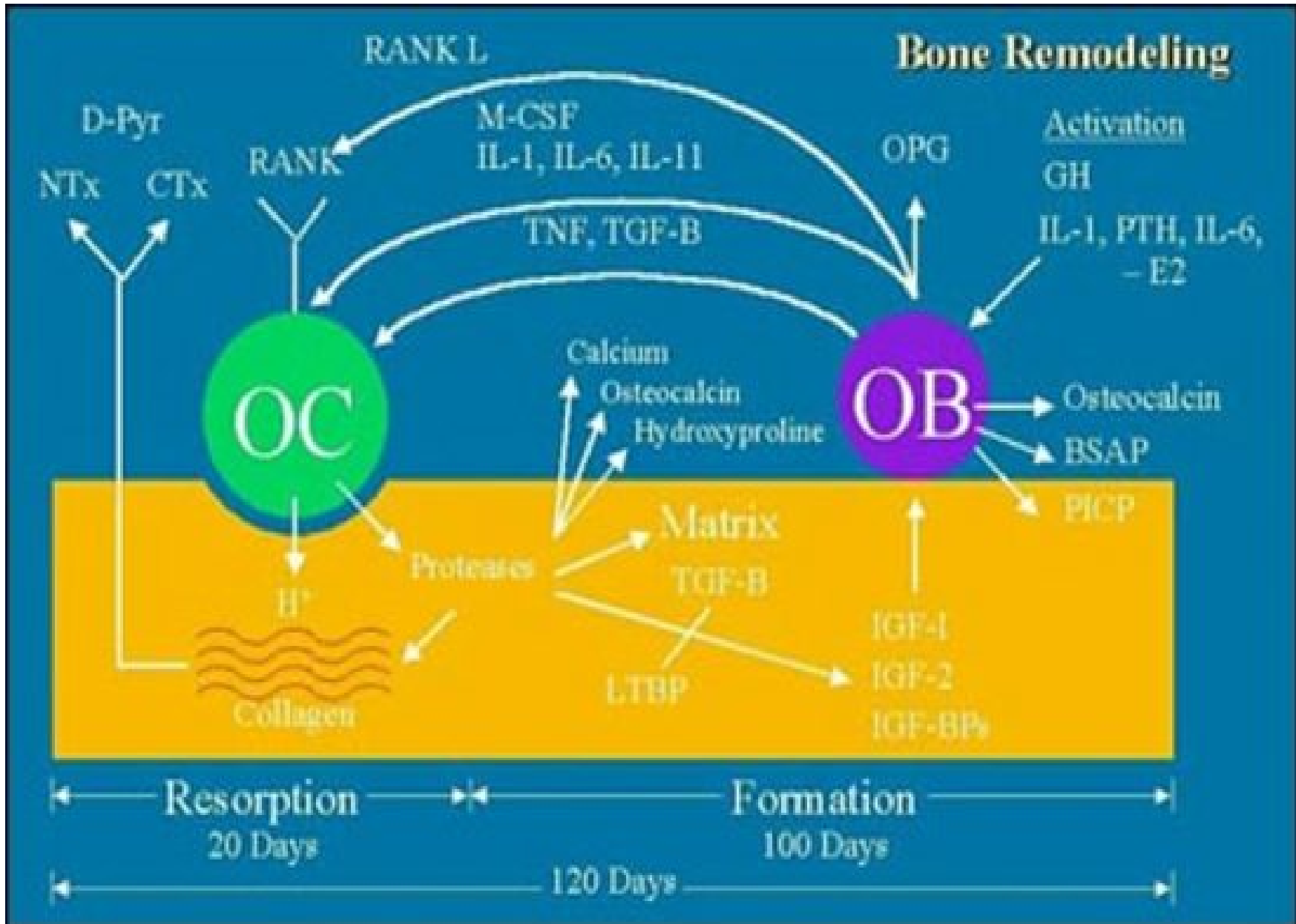


Active

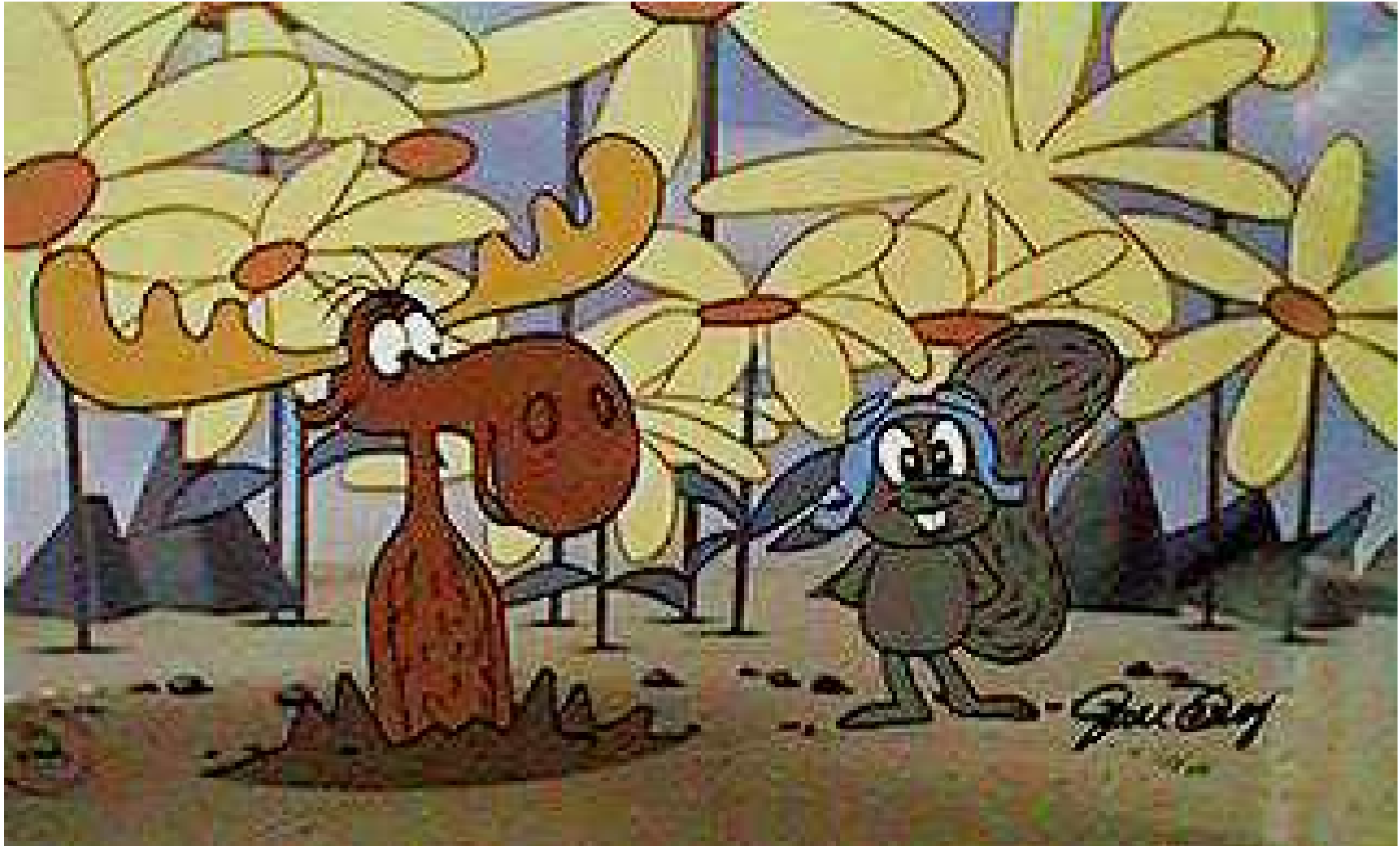


# Control of Osteogenesis

- **MMP**
  - Matrix Metalloproteinase9
  - In chondroclasts, degrades matrix
- **VEGF**
  - Required for vascular invasion, synthesized by hypertrophic chondrocytes, sequestered in ECM, and released when MMP degrades matrix
- **RUNX2/CBFA1**
  - Required for osteoblast formation
- **RANK/RANKL**
  - Osteoclast formation, activation
  - Stimulates differentiation of precursors
- **MCSF/c-FOS**
  - Proliferation of precursor cells
  - Commitment of hematopoietic precursors to adopt osteoclast fate (vs macrophage)

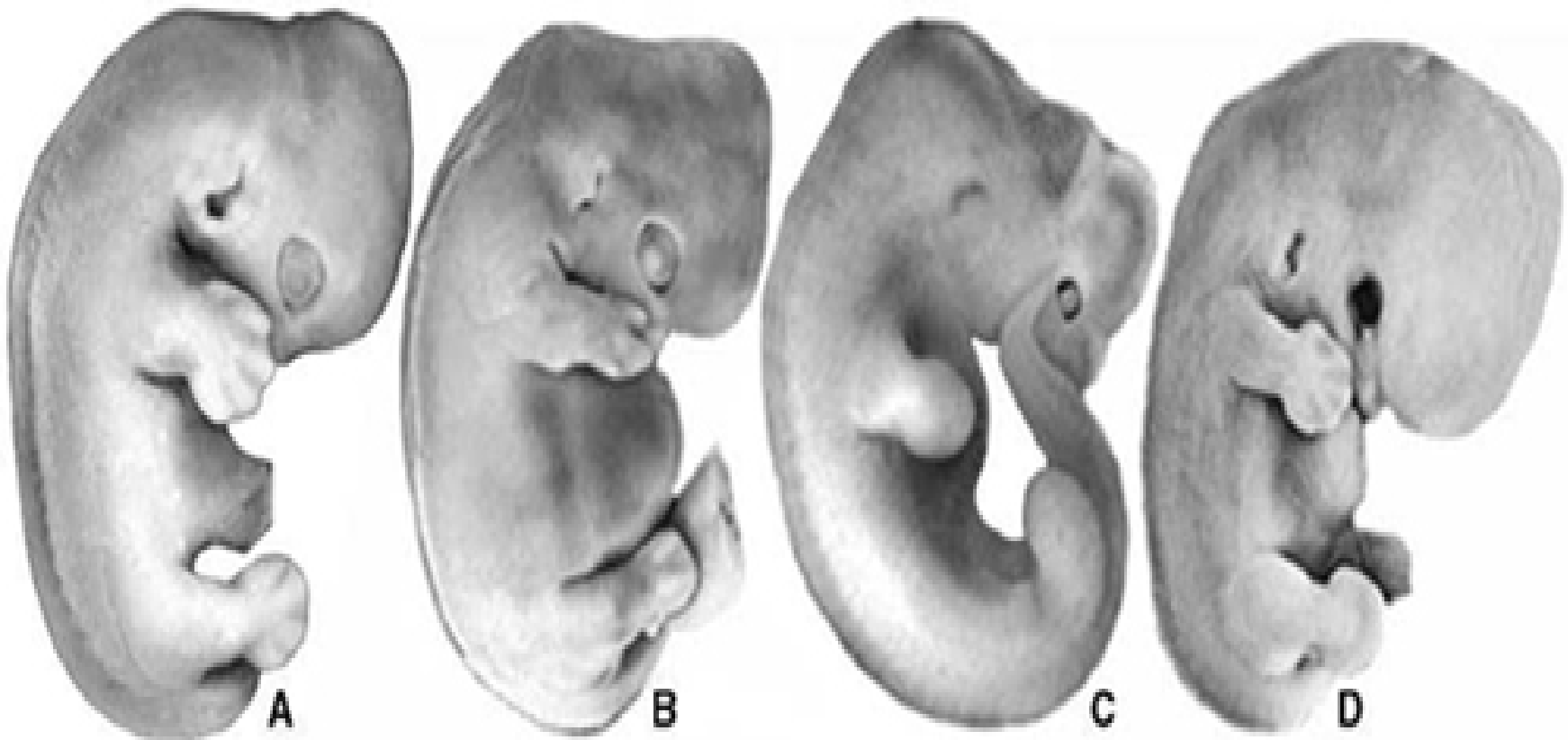


*And now for something you'll really like...*



# Chapter 10

- Which is cat, human, cow, horse?



# Chapter 10

- Chapter focus on growth of miniature limb to adult size (4 of 4)
  - Cohn and Tickle's 4 phases of development:
    - Initiation of bud
    - Specification of limb pattern
    - Differentiation of tissue and shaping of limb
    - Growth
- Diversity of form in adult, conserved early developmental processes
  - Prenatal
  - Postnatal
  - Timing
  - Genetic and Epigenetic

# Many interesting ways of tackling the issue..

- Morphology (R. W. Haines)
- Stereology and morphometry
- Biochemistry of the tissue and matrix
- *In vitro* studies of cells
- Regulatory pathways
- Pathology
- Zoology
- And oh, yes.....Paleontology!
- Some highlights:
  - *Fins to limbs and back to fins (Caldwell)*
  - *Limb loss in snakes (C & T)*
  - *Miniaturization (Hanken)*

# *What we don't know.....yet*

- What came first; the chicken or the l'egg?
  - Ahlbert
- Self-organizing mesenchyme?
  - Newman
- Digits: adaptation to terrestrial life or originate in water?
  - Laurin
- Did tetrapods walk *in* the water first?
  - Shubin
- Polydactyly?
  - (Coates and Clack)
- Critical: free fins from body axis, origin of limb axis OR digital arch hypothesis?
  - Tanaka
  - Coates

# *What we DO know*

- In evolutionary terms, cartilage and bone are ancient tissues.
- EO mechanisms are also ancient.
  - Advanced in fish
  - Passed on to Tetrapods
  - Shows immense diversity and specialization
- Rapid evolution possible due to independence of 'modules' (skeletal elements of the limb) yet underlying processes/properties unaltered
- Material properties also conserved
  - Changes in size and shape of skeletal elements



- 2 types of bone formation (both occur simultaneously—interact \*)
  - Perichondral (intramembranous)
    - Laid down on the outside/CT
    - Bone collar (constrains) + girth in diaphysis
    - Primary mechanism in early tetrapods
  - Endochondral
    - From inside, laid down on template/matrix
    - Allows for complex joints
    - elongation
  - As length increases, bone retains shape
    - Postnatal growth of OOM
    - Requires remodeling (\*)

# Epiphyses

- Growth in length occurs only at the ends of bone, and only until diaphysis ossified; then only growth in width, or modification of shape.
- Chondrocytes
  - 2 stage differentiation
    - Proliferation of cells (increase in #)
    - Terminal differentiation (hypertrophy)
      - Increase in cell size
      - Increased matrix synthesis
      - Leads to interstitial growth

- R. W. Haines
  - Extensive work in 1930's, 40's on structure of epiphyses
  - Determined conserved across tetrapod group

greater part of the calcified cartilage of the secondary centre are replaced by marrow and endochondral bone, so that the tissues of the shaft pass without interruption into the epiphysial region, and approach the articular surface. The remaining cartilage forms a thin layer of hyaline articular cartilage (Fig.

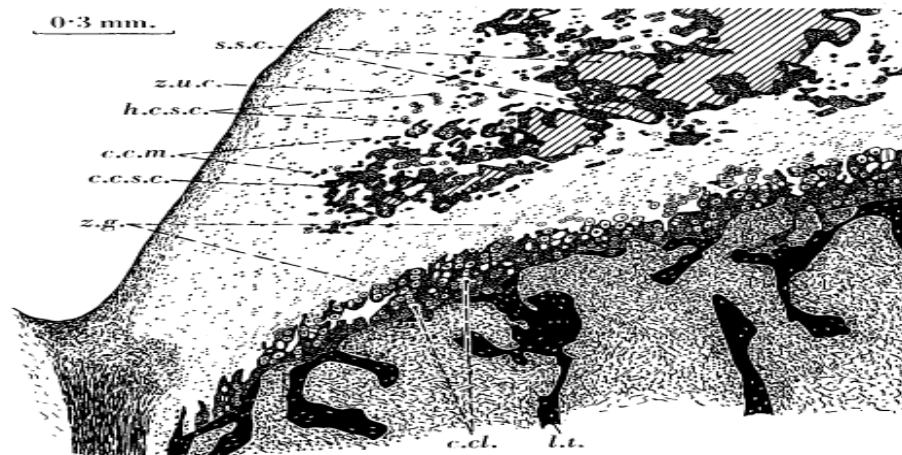
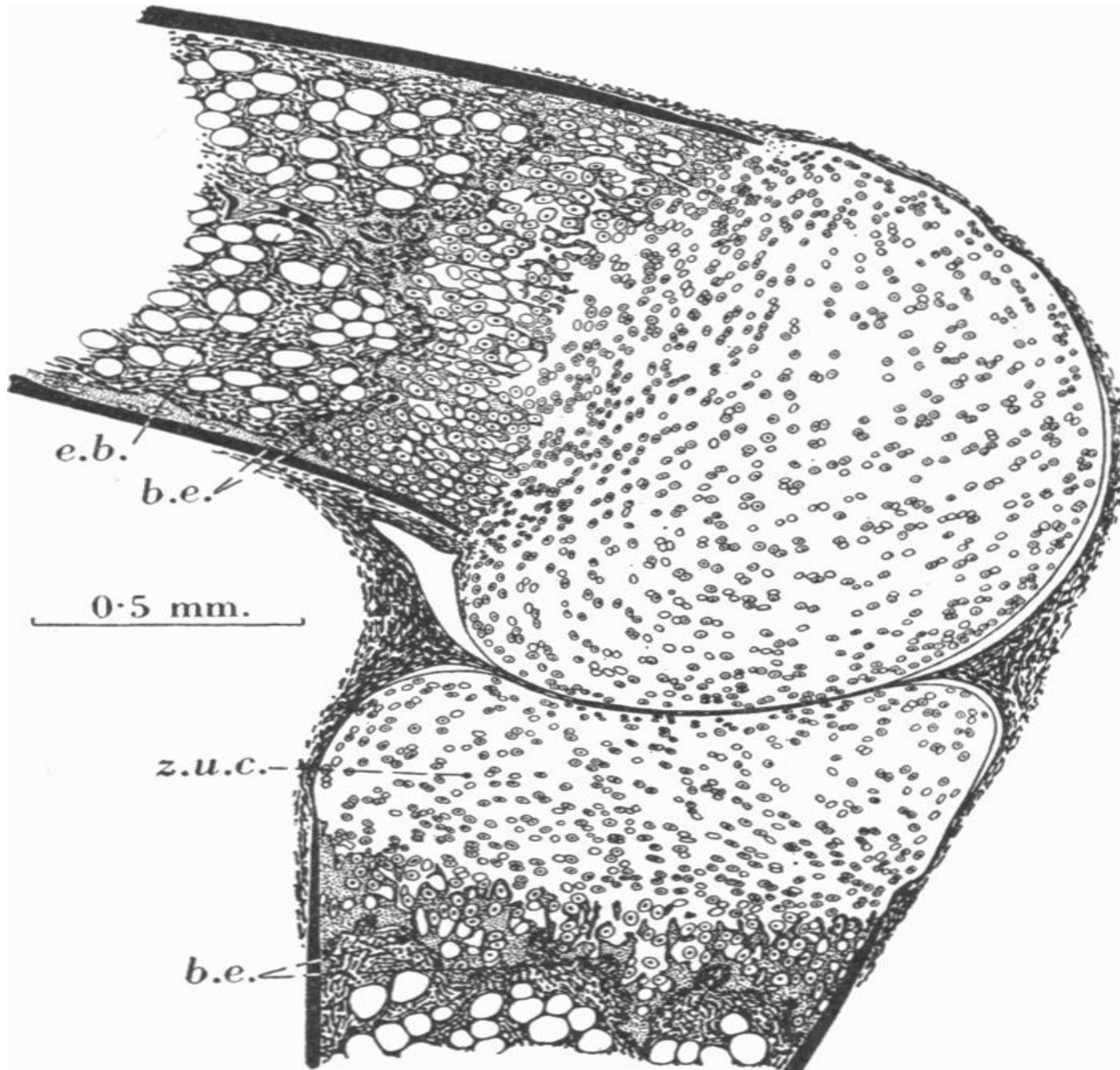


Fig. 4. Part of the lower end of the ulna. Same series as Fig. 2.

6, *a.c.*), beneath which a few unmodified cells represent the remains of the formerly wide zone of undifferentiated cells (*z.u.c.*). Passing towards the marrow the cells first become hypertrophied (*h.c.s.c.*), and then the matrix becomes heavily calcified (*c.c.s.c.*), this formation being derived from the uneroded subarticular part of the secondary centre.

In the shaft a cement line (*cem.*) separates the endosteal part of the endochondral bone (*e.e.b.*) from the periosteal bone (*p.b.s.*). The periosteal bone itself has now grown beyond the original limits of the shaft, and has come into contact with the cartilage of the epiphysial region (*con.*), the bone being sharply deflected by the cartilage. In the region of contact the cartilage has become calcified (*c.c.p.b.*).

# Epiphysis of young amphibian



# Epiphysis of young reptile

- Epiphysis is cartilaginous at this age; remains this way throughout growth in amphibians, may develop a secondary center of ossification in reptiles
- Chondrocytes only slightly organized; elongation is slow because only a few chondrocytes are aligned in the direction of growth.
- Subset of cells contributes to articular cartilage; the rest to growth and formation of bone within the growth plate

plete individual at University College leave no doubt as to the existence of secondary centres, the last confirming Albrecht's observations of separate centres for the vertebral spines. Further, I have been able to prepare sections of bones from four individuals, of 105 mm. total length (about 55 mm. snout-vent length) given me by the late Dr McMaster, of 149 mm. snout-vent length by courtesy of the Zoological Society of London and of two subadults, one sent by Prof. Gowland and one of 225 mm. from Prof. Appleton's collection.

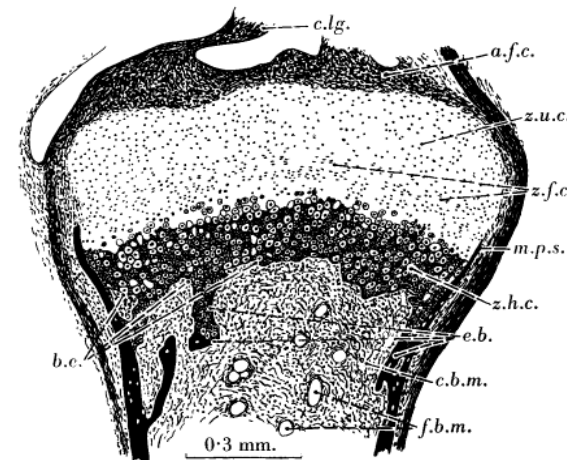


Fig. 1. Longitudinal section of the upper end of the tibia in a young *Sphenodon*. Total length 105 mm.

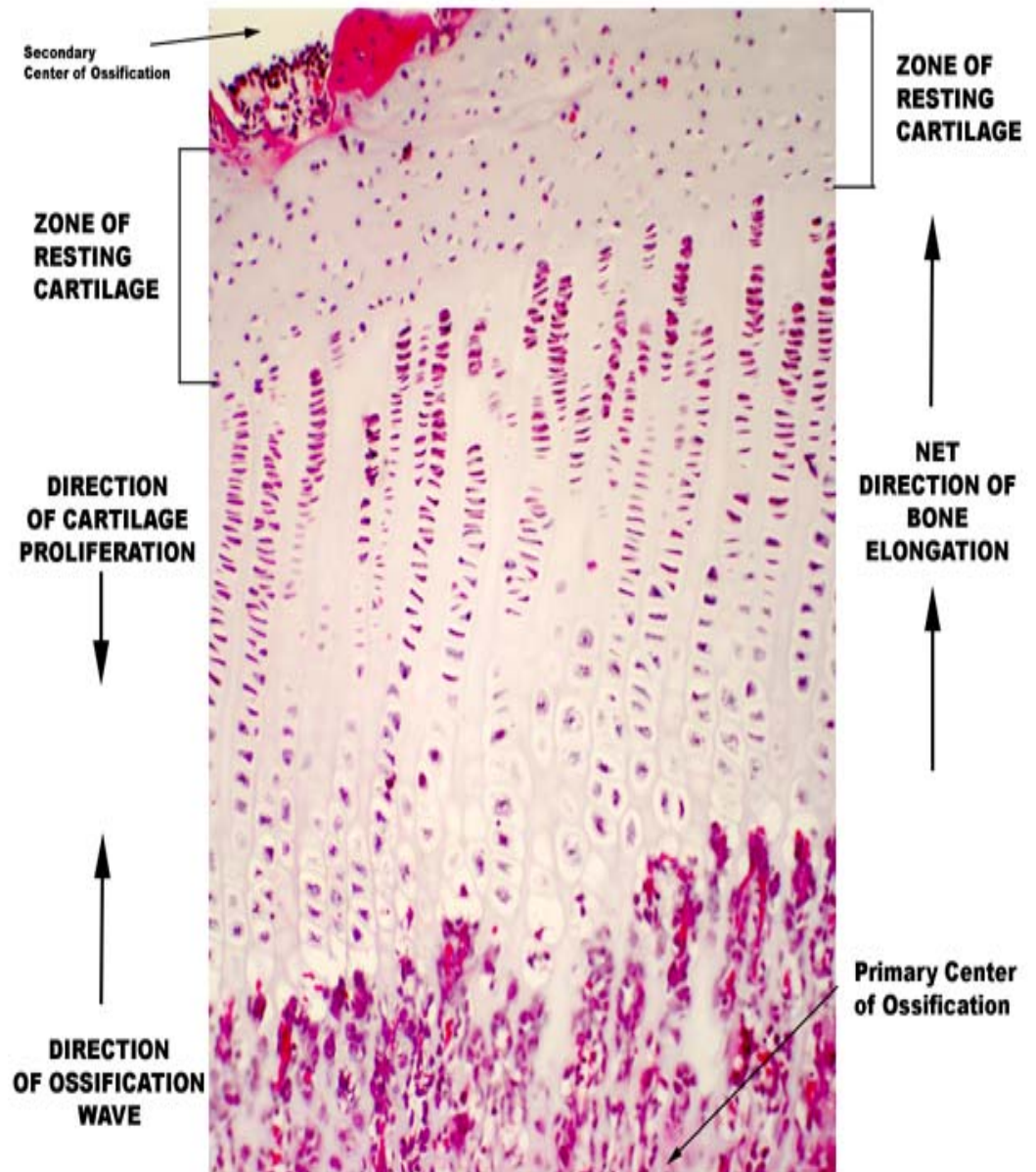
In the youngest specimen (serial of knee prepared), the essential epiphysal structure is well shown in the tibia but no secondary centres are yet developed. A thick articular fibro-cartilage (*a.f.c.*) covers the articular and non-articular surface, and gives attachment to the cruciate ligaments (*c.lg.*). The main mass of hyaline cartilage contains undifferentiated cells only (*z.u.c.*). At the level of the growing margin of the periosteal shaft (*m.p.s.*) this zone passes into the growth cartilage, a layer of flattened cells (*z.f.c.*) which is convexly bulged towards the articular surface. The zone of hypertrophied cells (*z.h.c.*) is well developed, and the calcification of the matrix is continuous, but the cells are as yet quite irregular in their arrangement. The surface facing the marrow is irregular, with large bays of erosion (*b.e.*) cut into the cartilage, indicating active destruction. Some endochondral bone (*e.b.*) has been laid down on the

- Compare the organization of the chondrocytes seen here with the two previous slides.
- Differentiation cascade clearly indicated.
- Growth much more rapid, many cells aligned in direction of elongation.
- (Similar to young rat)

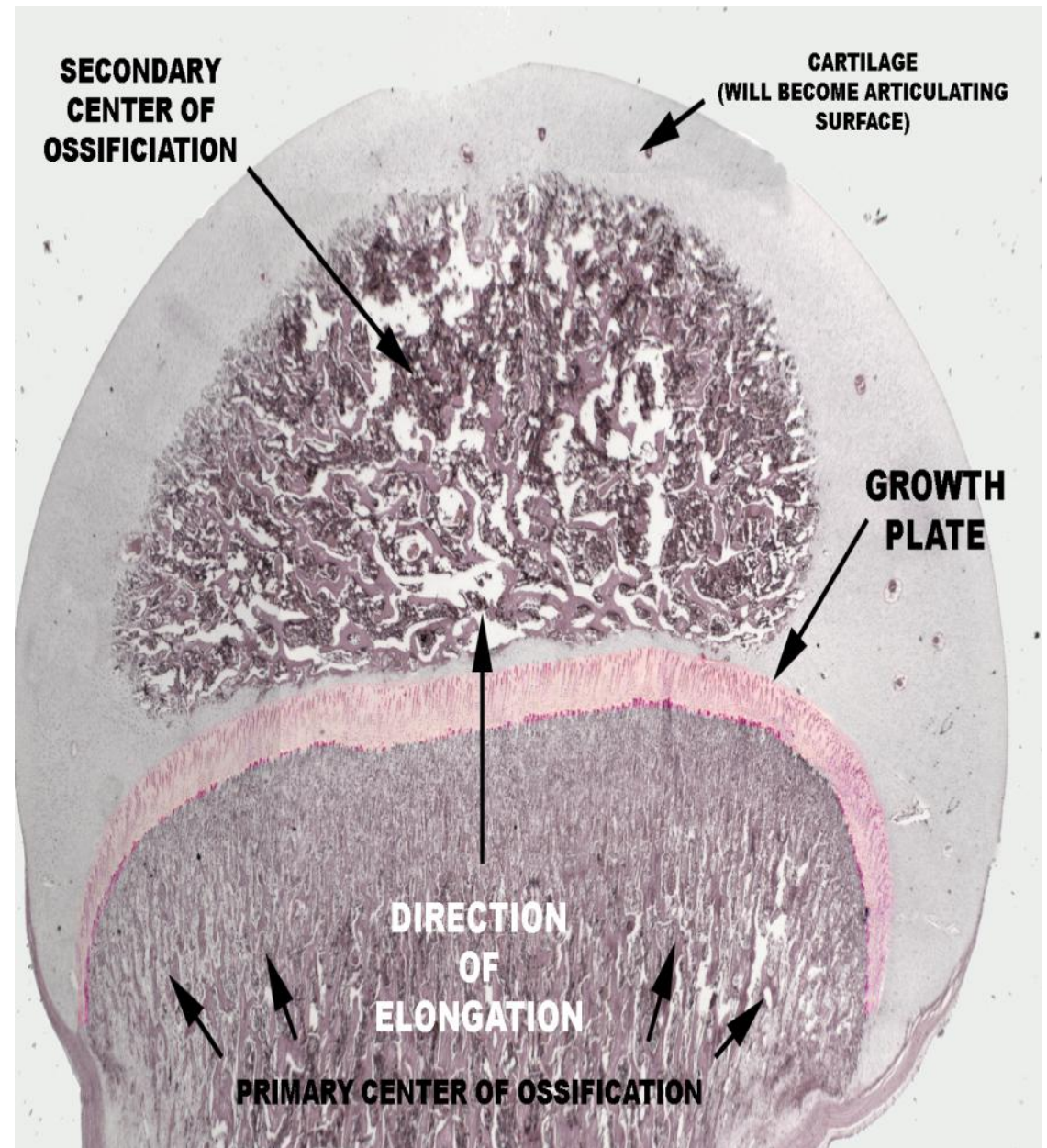


# Endochondral bone formation

- Proliferating cells small and flat.
- Hypertrophic cells larger, round.
- Formation of bone occurs as cartilage grows, and is replaced.
- Growth modulated by numbers of cells, rates of proliferation and cell death at junction of cartilage, bone.
- Bone formation lags behind elongation.



- Secondary ossification center separates articular surface from growth plate
- Articular cartilage
  - visco-elastic
  - load bearing
  - protective





- Epiphysis of reptile with secondary center of ossification. Compared to epiphyses of younger reptile, the chondrocytes are aligned into columns; more efficient interstitial growth

calcified cartilage by marrow spaces (*m.s.s.c.*), while on the remains of the cartilage endochondral bone (*e.b.*) has been laid down. In the zone of growth the cells lie in well-marked longitudinally arranged clumps, indicating active division, and in the zone of hypertrophy (*z.h.c.*) the cells form cartilage columns (*c.c.m.*). The bays of erosion (*b.e.*) and the endochondral bone have the usual reptilian arrangement.

This kind of epiphysial structure is probably found in all groups of lizards in which the secondary centres become ossified, with the sole exception of the Varanidae. This has been confirmed in Geconidae, Agamidae, Iguanidae,

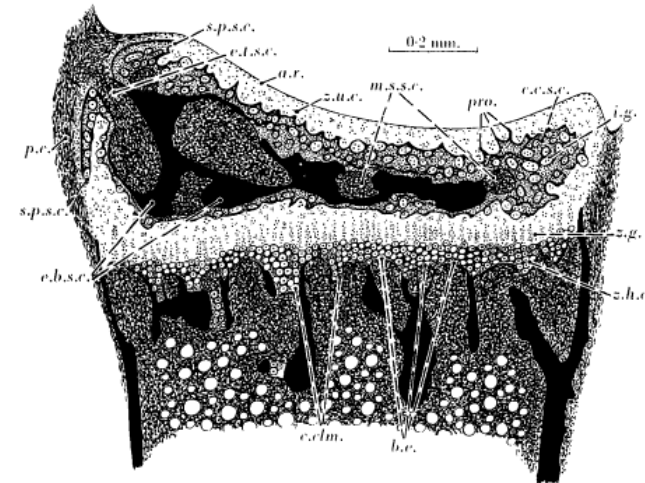


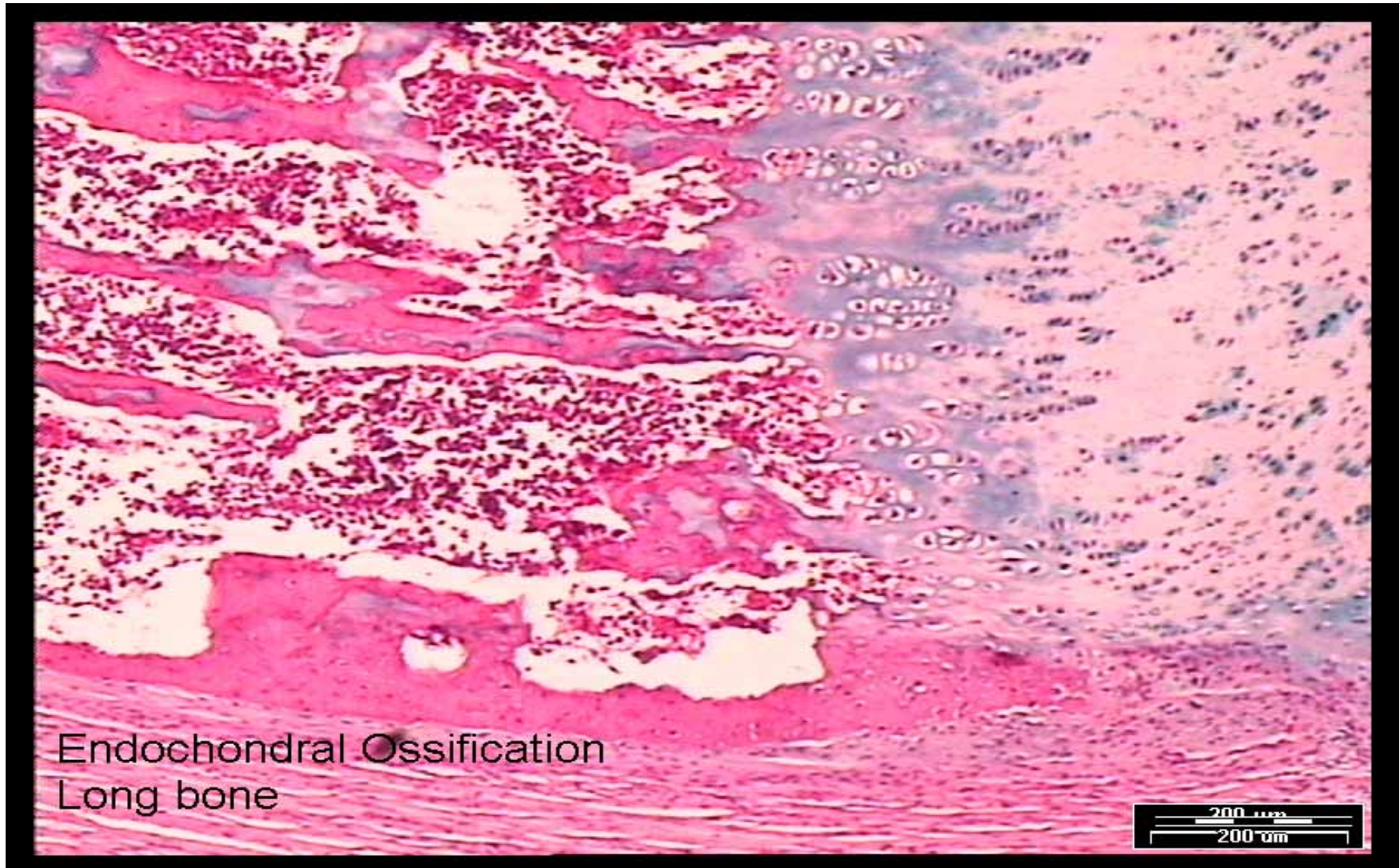
Fig. 1. Head of the radius of *Agama atra*.

Zonuridae, *Heloderma*, Tejidae, Lacertidae, Gerrhosauridae, Scincidae and Chamaeleontidae. The number of points of entry of the perichondral tissues is variable—usually one, but sometimes several. Again, in many epiphysial cartilages there are extra centres starting from intratendinous ossifications, e.g. the epicondyles of the humerus, the surface of the olecranon, the tibial tuberosity, the attachment of the cruciate ligaments to the femur, etc. Finally, as in mammals, some of the cartilages of the metacarpals and phalanges are ossified by extensions from the shaft, instead of by separate secondary centres (Heidsieck, 1928). But the type of ossification in the large limb bones is remarkably constant.

# Growth plates in mammals

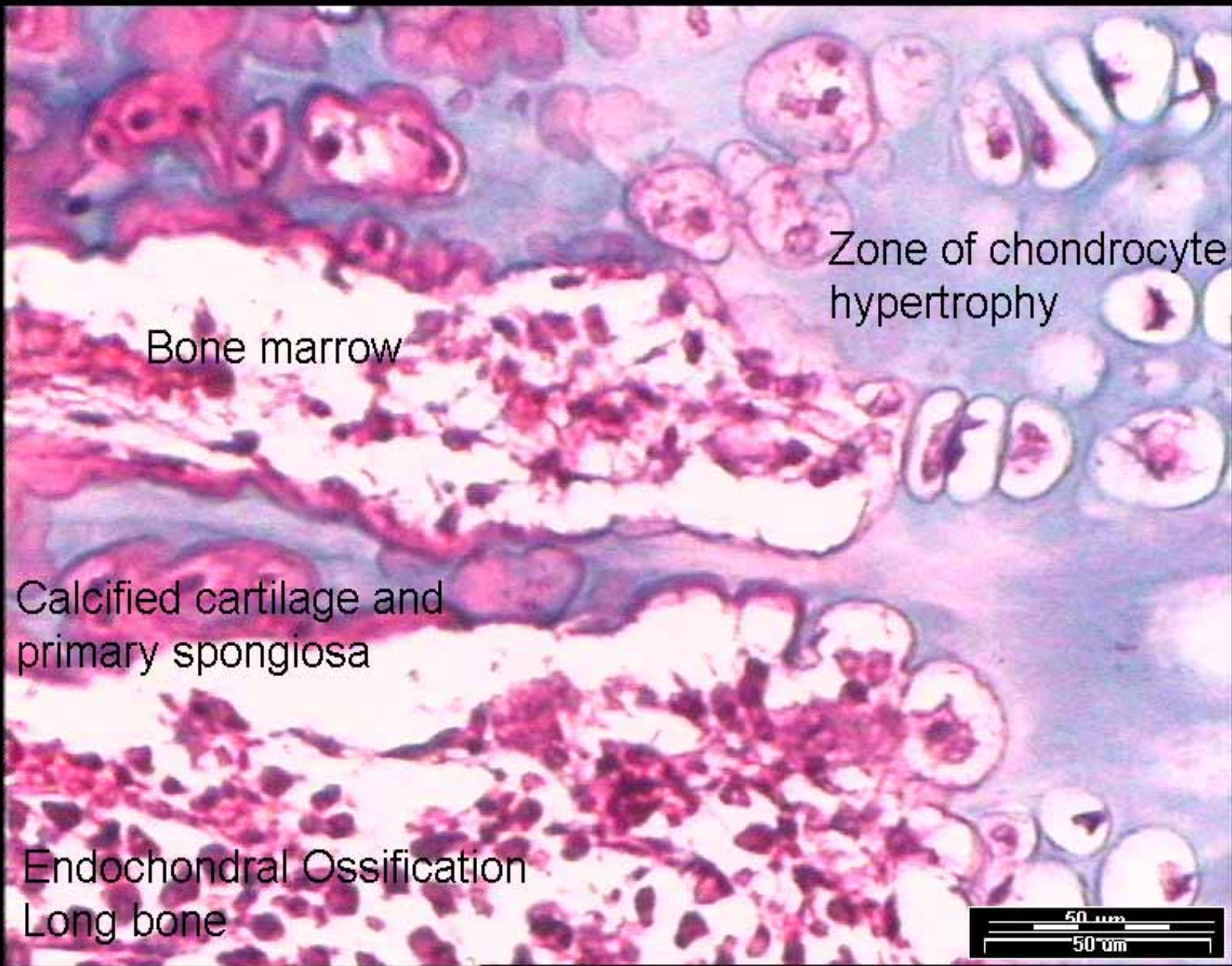
- Cells in columns aligned in direction of growth between secondary center and metaphyseal bone.
- Secondary ossification centers
  - Evolved later than epiphyses
  - Support in terrestrial environment? (Haines)
  - Birds appear to have secondarily lost this structure (large birds may have them, and many birds have it in the proximal tibia)
- Dual blood supply
  - Epiphyseal is primary nutrient vasculature
  - Metphyseal important in signaling cartilage/bone replacement.
- Zones can be defined
  - Resting
  - Proliferative
  - Hypertrophic

# Growth plates in mammals



Endochondral Ossification  
Long bone



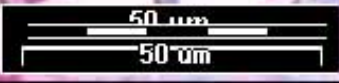


Bone marrow

Zone of chondrocyte hypertrophy

Calcified cartilage and primary spongiosa

Endochondral Ossification  
Long bone



Primary Center  
(Diaphysis)

Growth plate

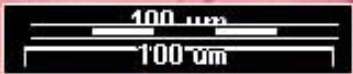
Secondary Center  
(Epiphysis)

Endochondral Ossification  
Long bone





Growth Plate  
Endochondral Ossification



# Tension physes

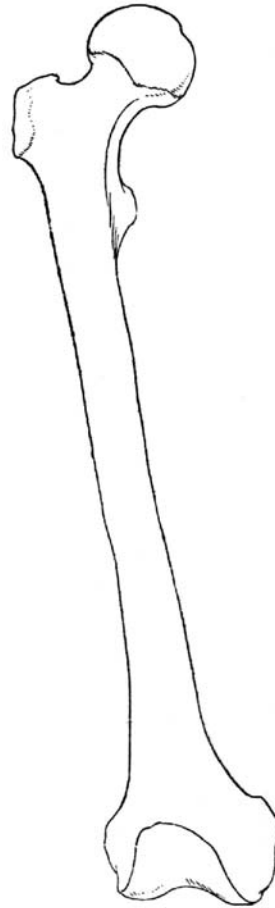
- Bony prominences associated with large muscles may have secondary centers of ossification (maintain EO)
- Often occur where tendon of large muscle group attach proximally on bone
  - Example: tendon of quadriceps femoris to tibial tuberosity
  - Tendon of supraspinatus to greater tubercle of humerus  
(I just like those words.....)
- Did these arise from sesamoid bones?
  - Small bones subjected to stress from tendon (patella)
  - Reptiles may have an ulnar patella
  - CT can ossify under differing conditions
  - Haines
  - Dr. Sumida's note: this hypothesis no longer supported.



# Bone, the organ

- Structural support
- Muscle attachment
- Protection
- Calcium and phosphorus
- Mineralization of cartilage to provide scaffold for osteoblast activity
- Matrix secreted by hypertrophic chondrocytes provides microenvironment for immune cell maturation (function even after cells that synthesized the matrix have died).
  - Collagen X
- Hemopoiesis
  - Hematopoiesis
  - Immune cells

- Genotype
- Hormone
- Embryonic
- Multiple rhythms
- Paracrine/Autocrine



Biomechanical

Nutrition

Disease

Drugs

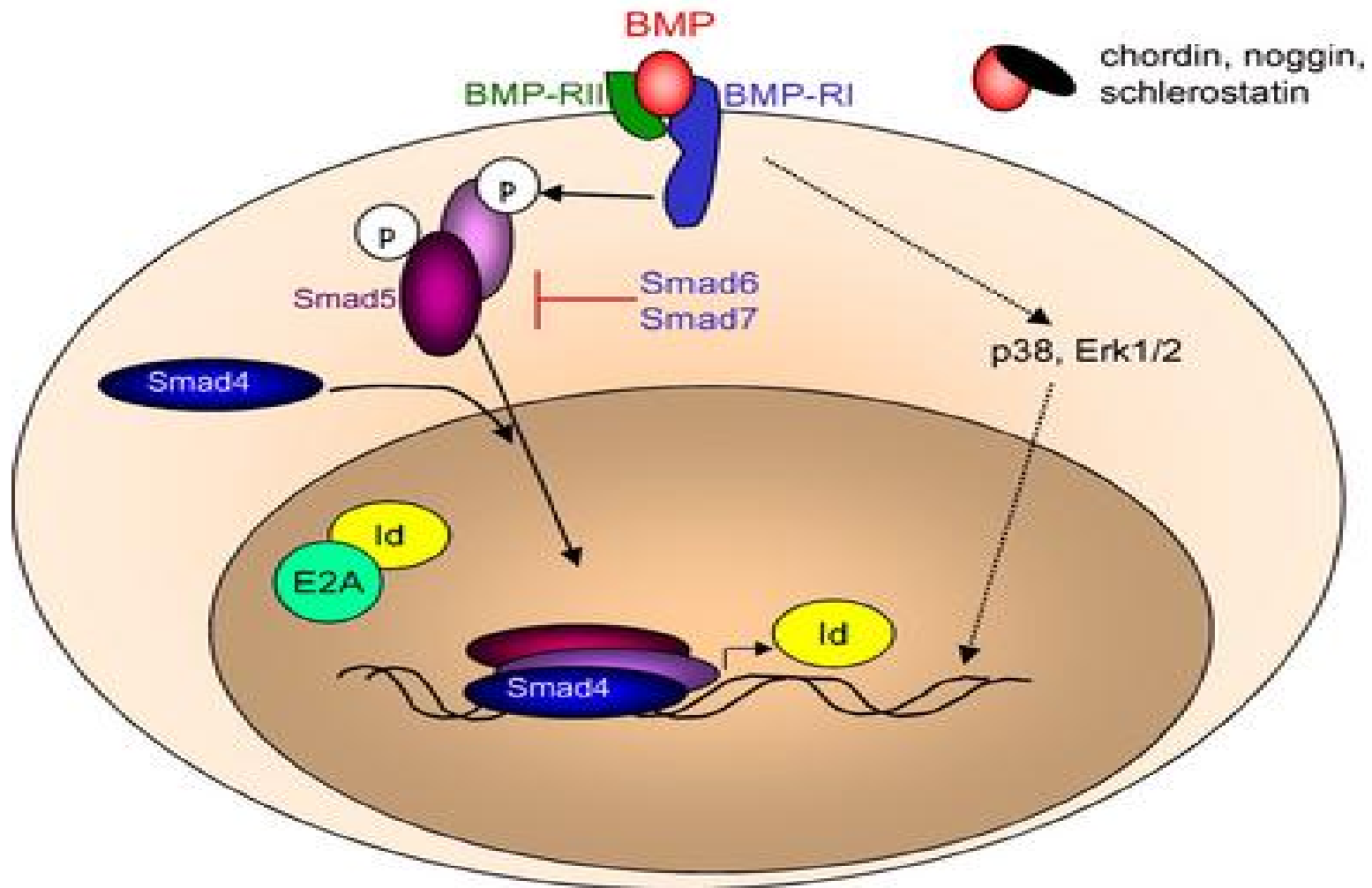
- Cartilage grows by intrinsic factors
- Bone grows by extrinsic factors
- Rate and duration of EO (and variations in form of limb or fin) influenced by many factors
- Patterning
  - Early development
  - Postnatal?
    - Homeobox genes—pattern, shape, identity of elements (sufficient to explain diversity?)
    - How many stem cells, or divisions?
    - Directionality?

# BMP-5

- Mouse model
- Mutagenized mice bred with recessive mutant mice (short-ear—specific changes in mouse skeleton: size, shape and number of bones. Gene required for normal growth—deletion mutants viable, fertile, show skeletal defects against normal background)
- Encodes gene for BMP-5 (family of factors with multiple regulatory effects on development, affected dorsal/ventral axis formation, L/R symmetry, growth, differentiation and death of chondrocytes.

Kingsley

# BMP signaling pathway



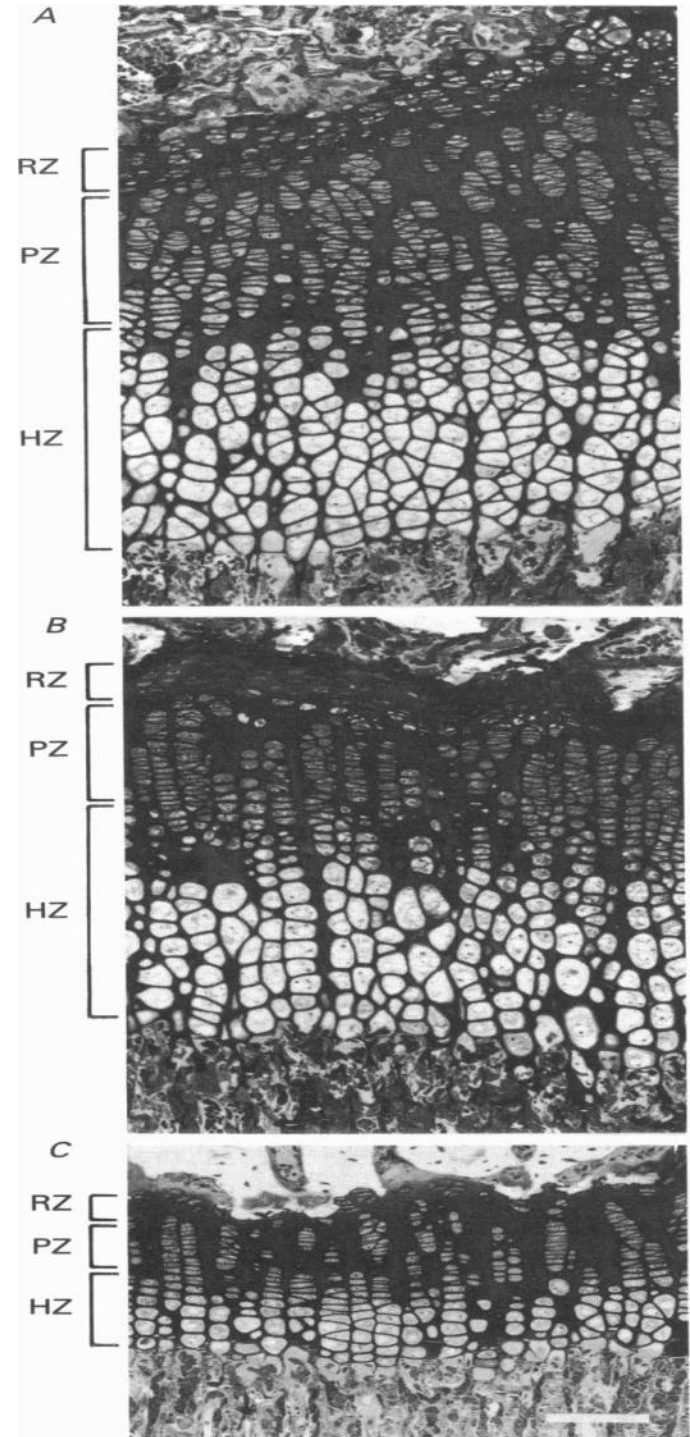
# Growth strategies

- Different mix of 3 basic cellular activities allow for varying rates of growth (all present in EO in early tetrapods)
  - Rapid growth based on hypertrophy in rat
  - Rapid growth based on proliferation in chick
  - Slow growth based on matrix synthesis
- Matrix components conserved
  - Collagen
  - Proteoglycan
  - MMP's
- Change in size by change in duration and/or rate of growth
- Dinosaurs versus giant crocodiles
- Variation in mammals (developmental stage, maturation rate)
- Mice bred for increasing tail length (Rutledge)
  - Increase in number of vertebrae
  - Increase in size of individual vertebrae

- **Determinate growth**
  - Ceases, not resumed (closure of growth plate)
- **Indeterminate growth**
  - Never ceases, may slow
  - Epiphysis remains cartilaginous
  - No bony union between epiphysis and diaphysis
  - In fish, amphibians, reptiles, the epiphysis may not develop a secondary center of ossification, articular surface is not separated from bone
- **Elephant**
  - Epiphyses open, growth throughout life
  - Dr. Sumida's note: paedomorphy?
- **Differential growth**
  - The two ends of a bone grow at different rates and/or duration, final contribution from each may be almost equal
  - Radius of dog: 60% distal, 40% proximal
  - Ulna of dog: 100% from distal end (proximal forms olecranon)
  - Postnatal effect
  - For most species, proximal humerus, distal femur contribute most

## Differential Growth

- Growth plate of proximal tibia in the rat
- Comparison of growth rates at different ages.
- Approximate growth:
  - 21 days: 275  $\mu$ /day
  - 35 days: 330 $\mu$ /day
  - 80 days: 85 $\mu$ /day
- Hunziker





# Differential growth revisited

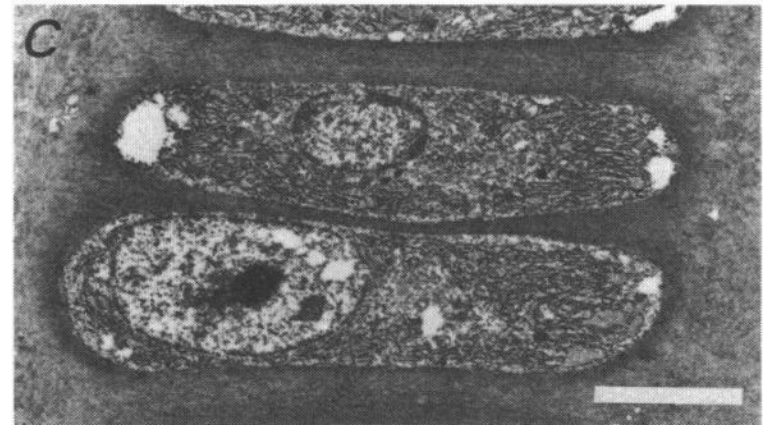
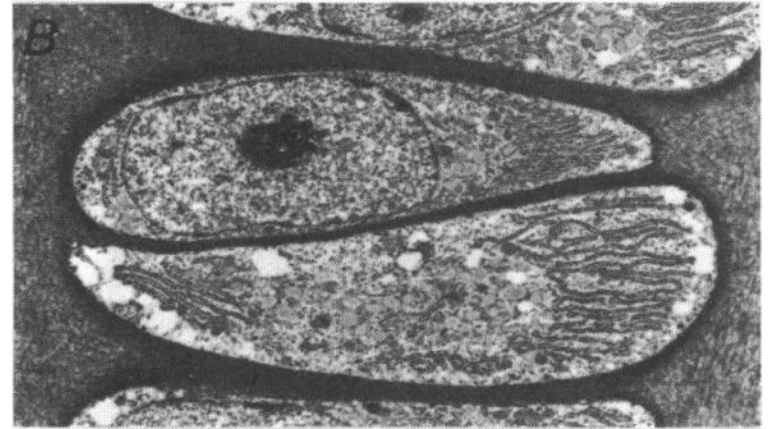
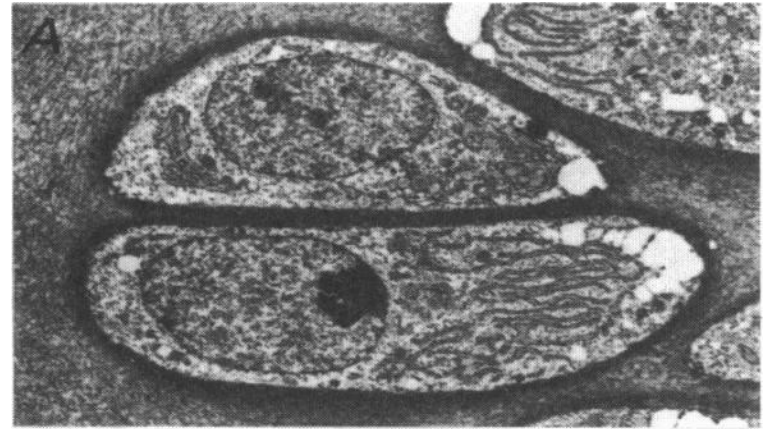
- Four week-old rat
- Four growth plates analyzed
- Proximal tibia--396 $\mu$ /day
- Distal radius--269 $\mu$ /day
- Distal tibia--138 $\mu$ /day
- Proximal radius--47 $\mu$ /day
  - At all rates of growth, contribution by both proliferative and hypertrophic chondrocytic zones.
  - At all rates of growth, contribution by matrix, but more significant at slower rates
  - More growth occurs during hypertrophic phase
  - Faster growth results in increased volume and height increase of hypertrophic cells

# How does differential growth occur at multiple growth plates?

- Growth plates may grow at different rates due to volume and height change of hypertrophic cells
- Shape change is as important as volume change (increased volume is translated into height increase, in direction of growth)
- Proliferation maintains steady state population of chondrocytes; numbers change as growth rates change.

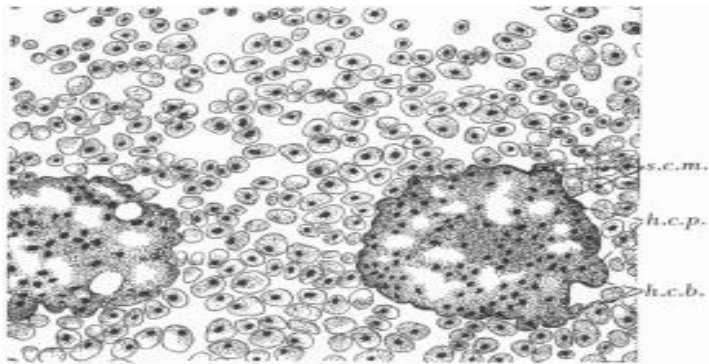
# Rats! More tibias..

- Proliferative cells from proximal tibial growth plate
- 21 days-A
- 35 days-B
- 80 days-C
- Height change greater in younger animal

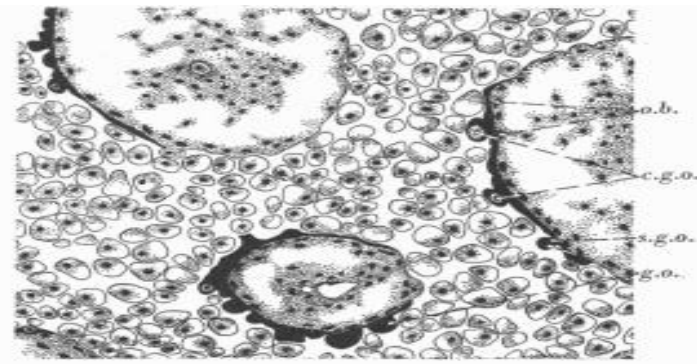


# Upper tibia of a baby crocodile

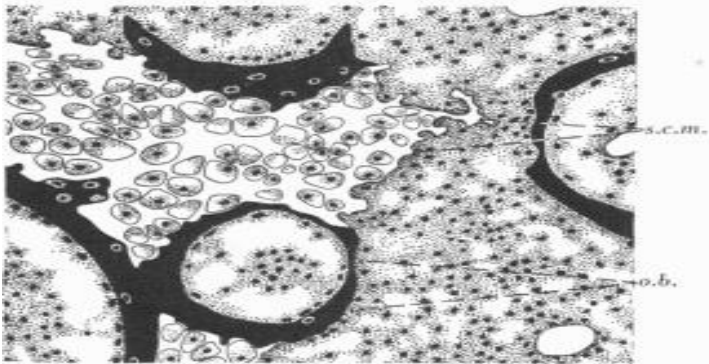
## Haines



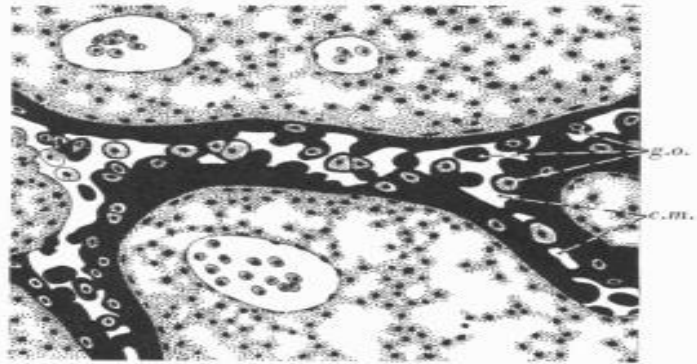
A



B



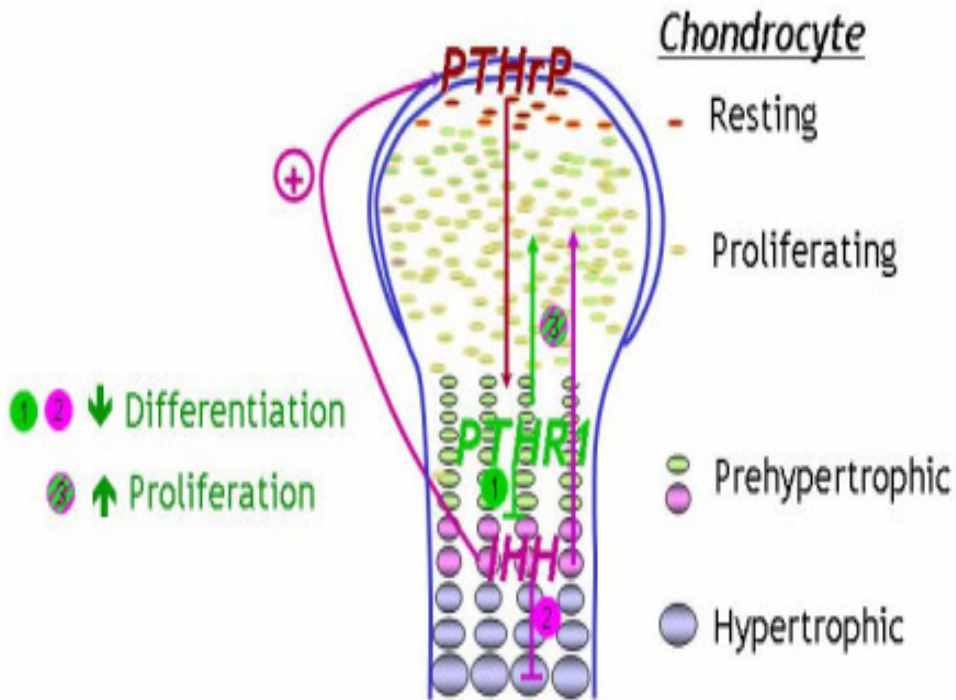
C



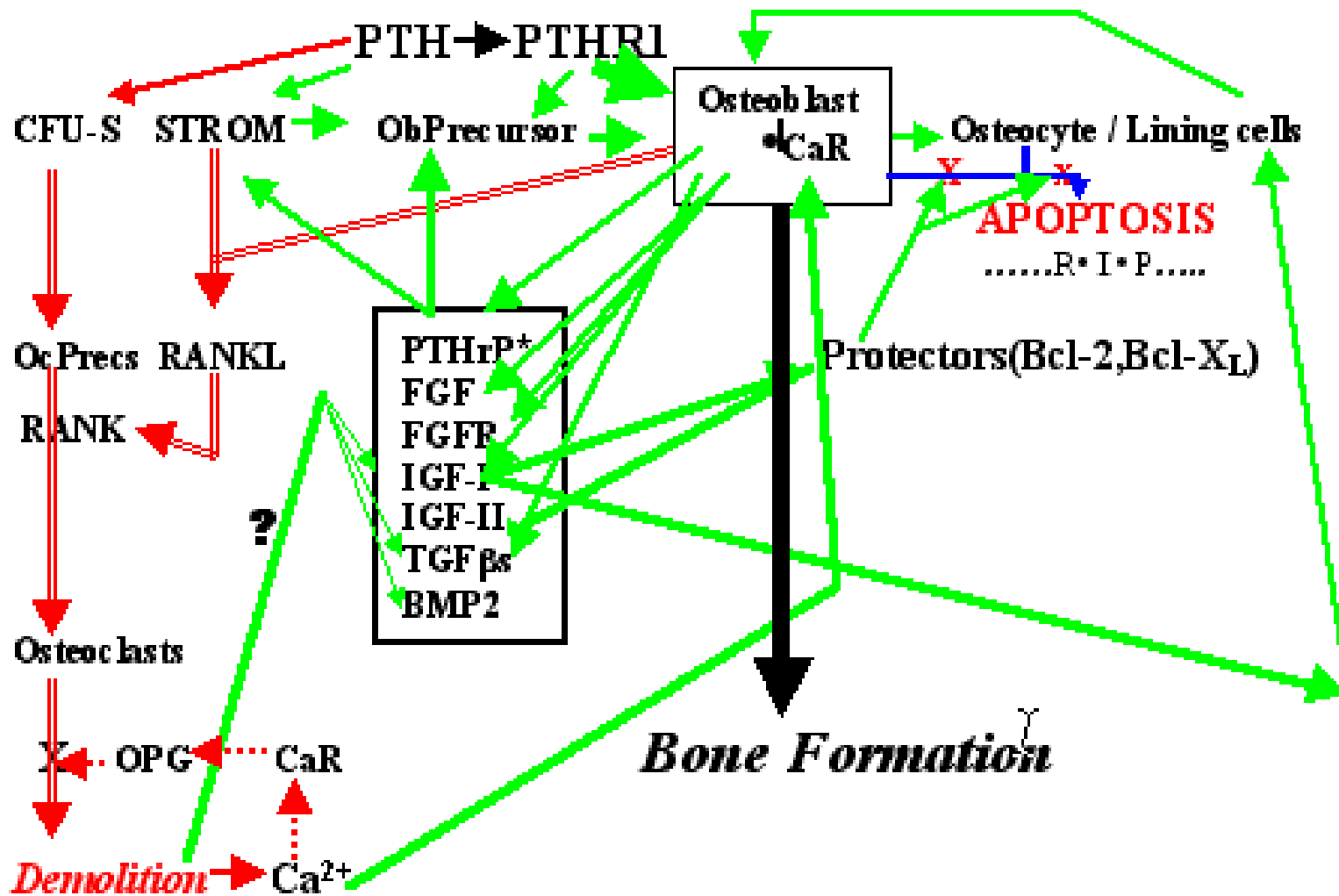
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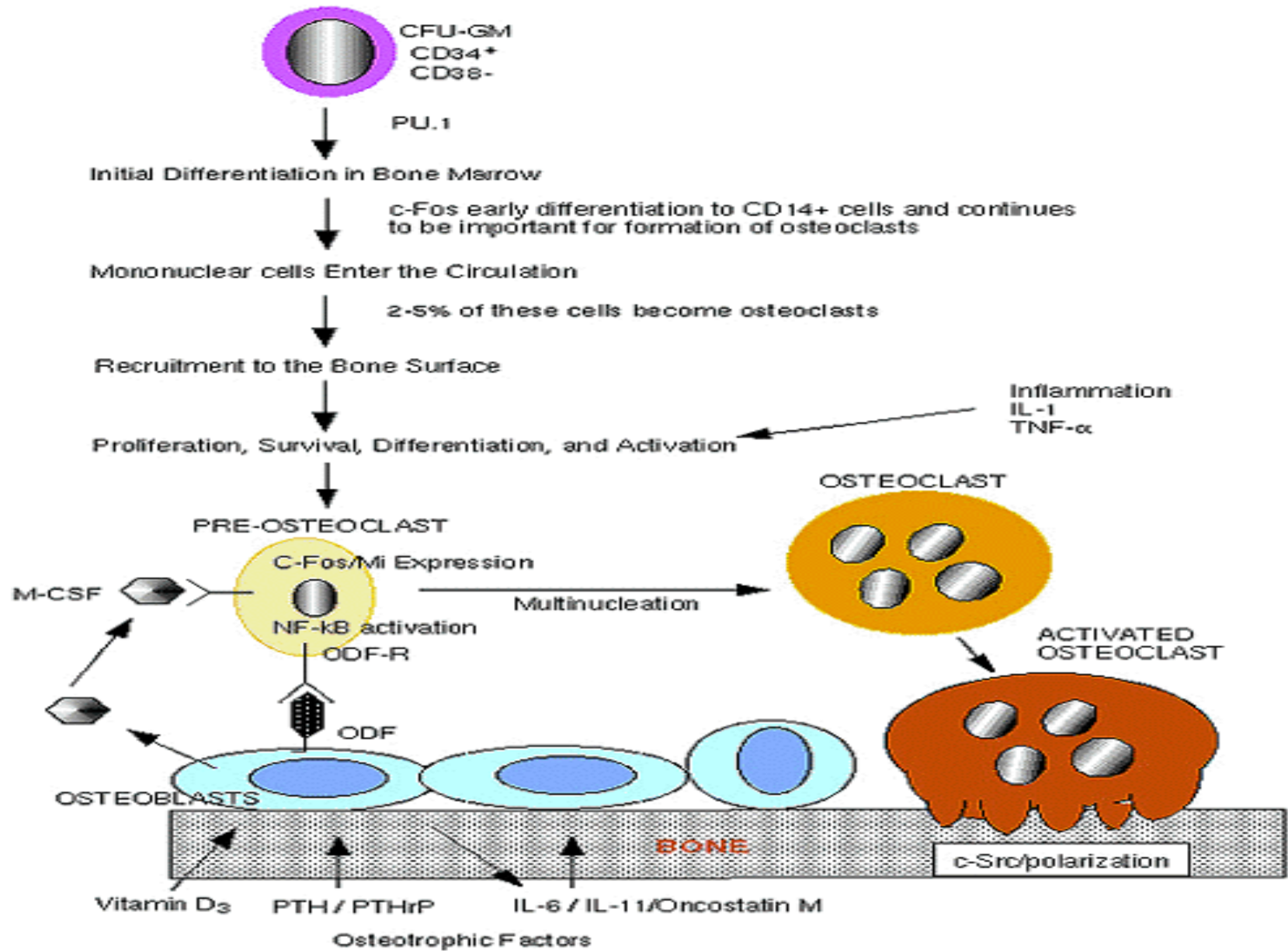
# Transitions

- Transition 1: Initiation of clonal expansion
  - Change in size, shape, and columnation of cells
  - *Sox* and *Hox*
- Transition 2: Proliferation to Hypertrophy
  - Proliferation ceases, chondrocytes begin to increase cell volume, change shape
  - IHH and PTHrP
- Transition 3: Chondro-Osseous Junction
  - Apoptosis (de-differentiate, adopt osteoblast fate?)
  - Endothelial and osteoprogenitor cells invade
  - Formation of bone by osteoblasts
  - VEGF, MMP's, *cbfa1* as well as IHH/PTHrP
  - Swine proximal tibial growth of 140 $\mu$ /day; 5.4 hypertrophic chondrocytes lost per column/day; each chondrocyte approximate 4.5 hour as terminal cell, 1 hour of that in condensed (apoptotic) form.

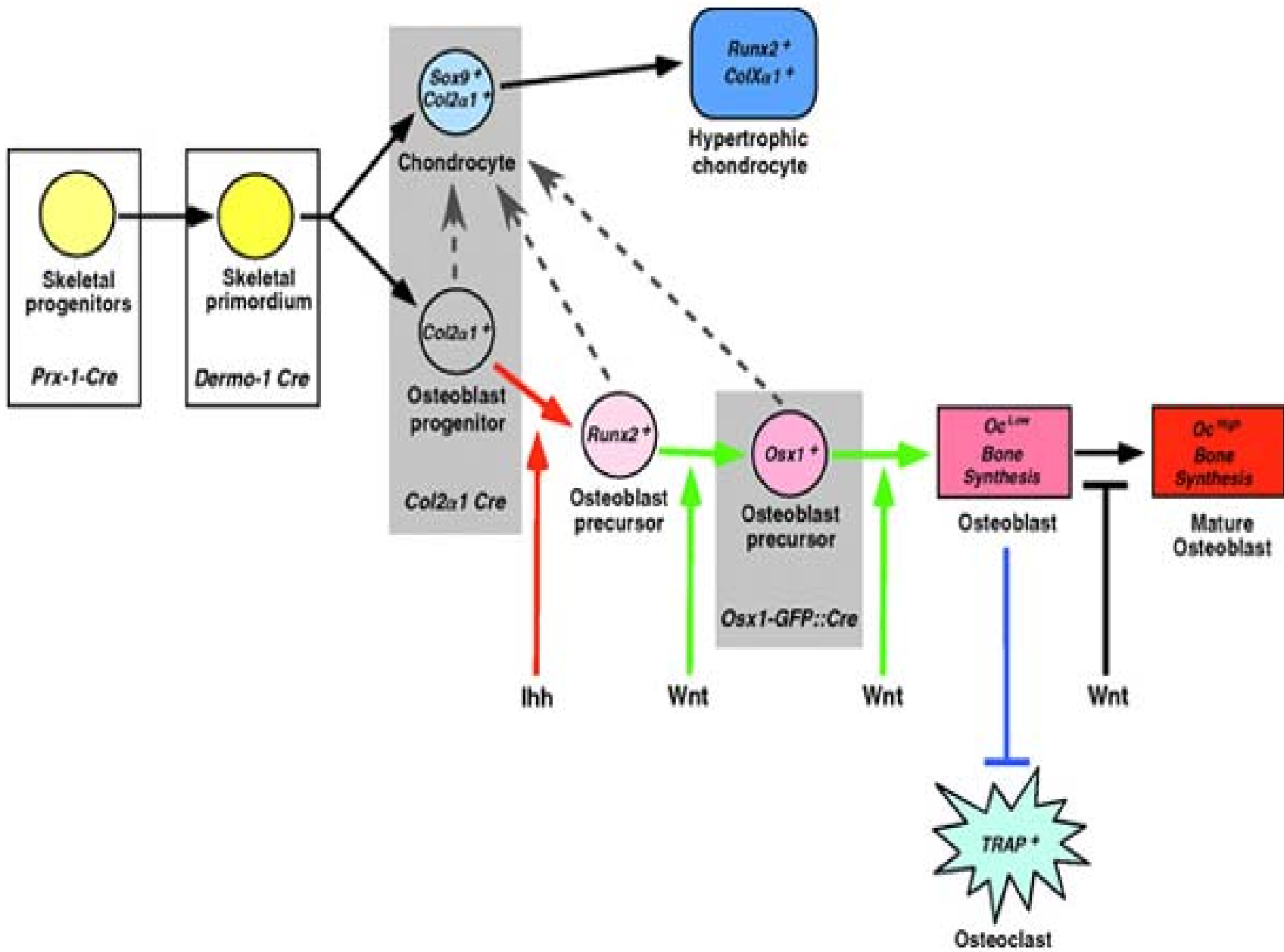


- IHH produced by hypertrophic chondrocytes, interacts via *Patched* in periosteum, initiate PTHrP production by perichondral cells—this interacts with a receptor on growth plate chondrocytes to delay maturation IHH also has feedback loops to proliferative cells and osteoblast/clasts









# Additional regulation

- FGF (Fibroblast Growth Factor)
  - Roles at all stages of skeletal development
    - Position/outgrowth of limb bud
    - Patterning of limb elements
    - Control of chondrocytic cascade
  - Master autocrine/paracrine regulator during postnatal growth
- GH/IGF (independent, direct FX as well)
  - Master systemic regulator of bone elongation (in mammals)
- Thyroid Hormone
- Glucocorticoids
- Steroids
  - Estrogen and androgen increase bone elongation
  - Estrogen required for epiphyseal closure

# Hypothalamus And Growth

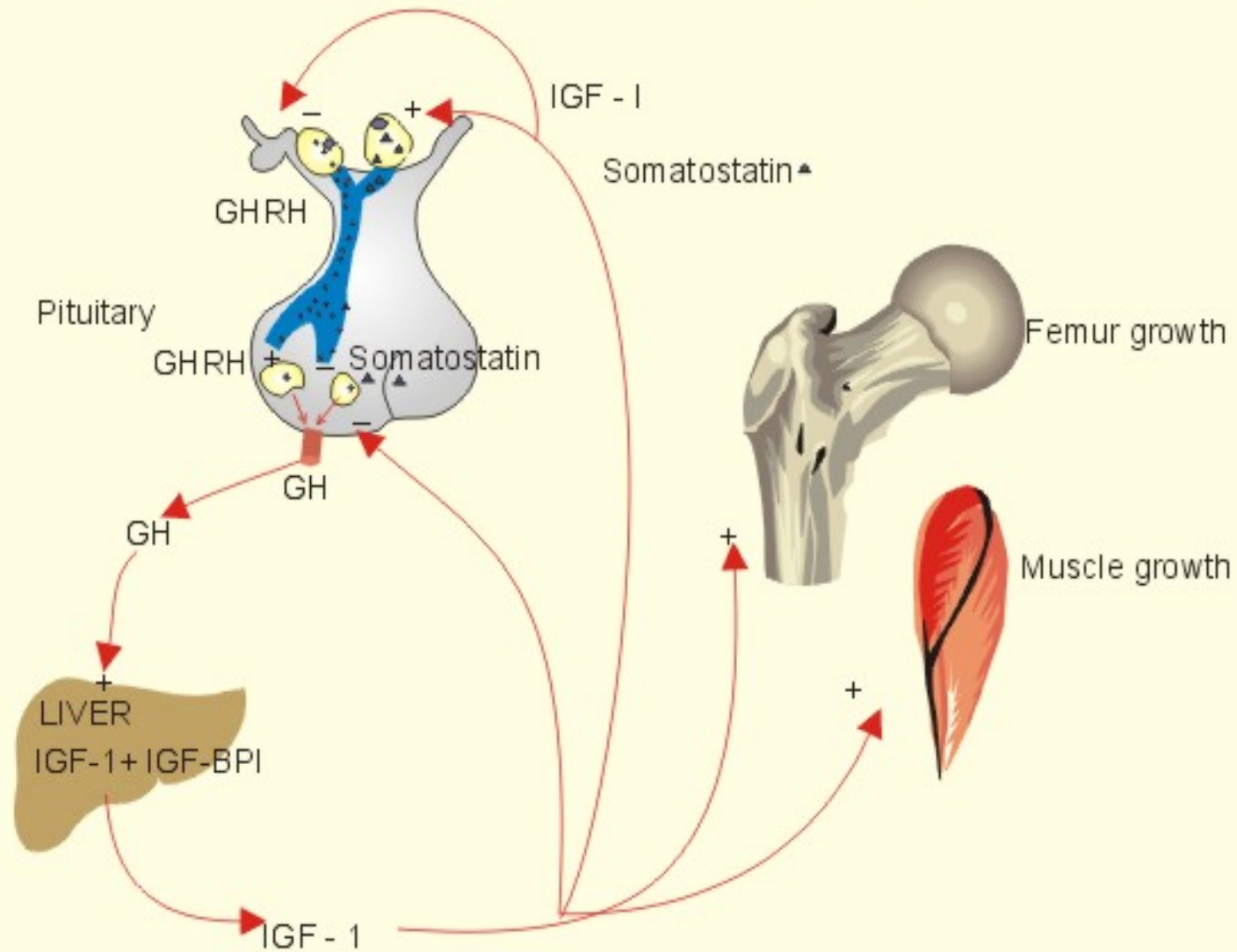


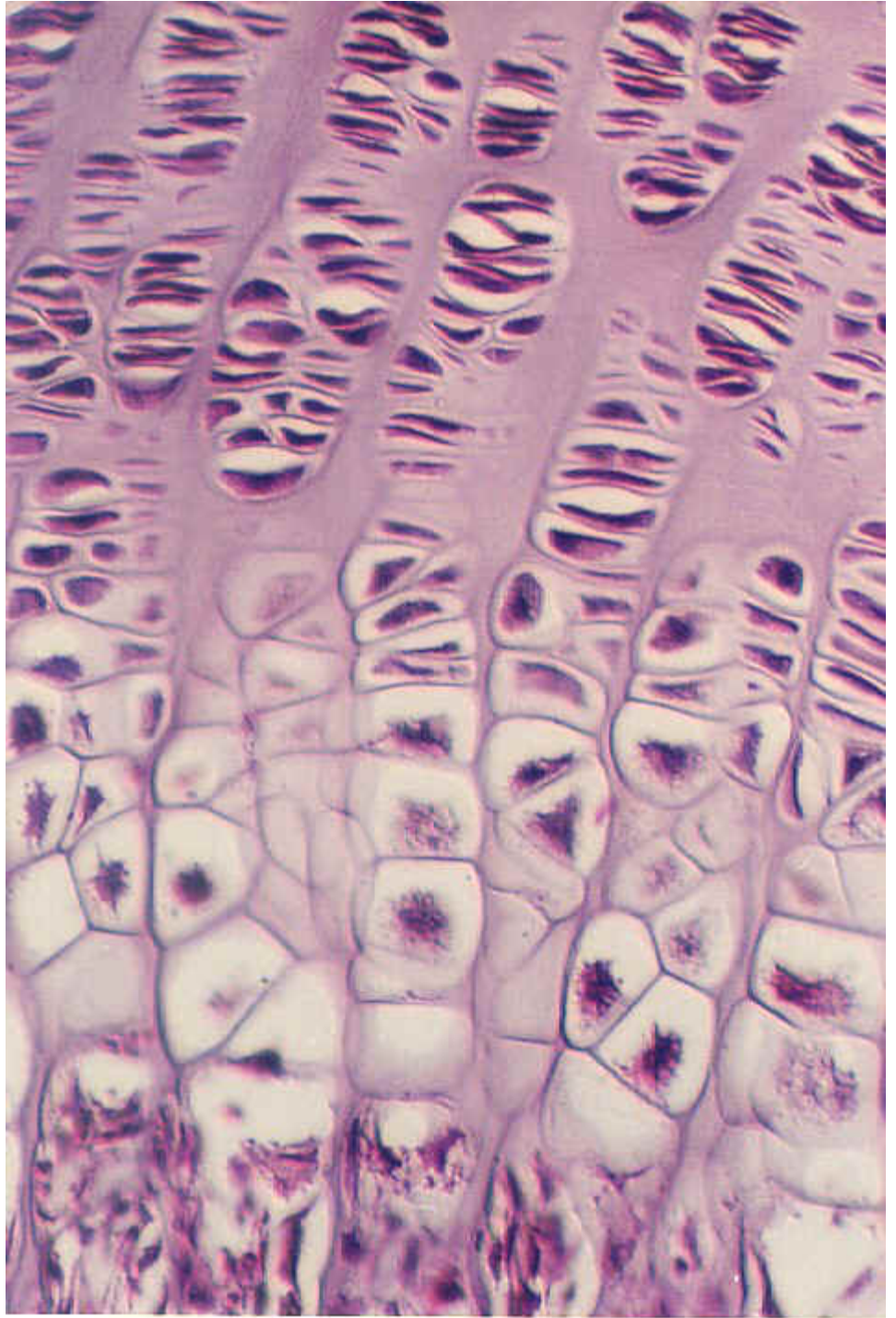
Fig. 30-1

# ECM

- Matrix in growth plate also has 3 zones
  - Pericellular, surrounds each chondrocyte
    - Interface with ECM
  - Common territorial
    - Route of epithelial invasion at C-O junction
  - Interterritorial
    - Separates different clonal expansions
    - Calcified in distal Hypertrophic Zone
- Proliferative Zone
  - Higher ratio of matrix to cells
- Hypertrophic Zone
  - More matrix is produced per cell (3X)
- Both zones contribute to elongation
  - Pericellular/territorial matrix volume contributes more than interterritorial
- Shape change (changes direction of long axis of cell; aligned with growth)

# ChChChChanges

- Shape change may be dependent on structural properties of interterritorial matrix; cells change in shape as increase in volume, direction of long axis aligns with direction of growth
- Like plants?
- Buckwalter



# Scottish Deerhounds

- Pseudoachondroplasia
  - Volumes of hypertrophic cells same in these dogs and normal dogs
  - Growth plate ECM disorganized
  - Proliferative and hypertrophic cells rounder than normal
  - Decreased differential height change for these two chondrocytic types accounted for all of the decreased bone elongation seen in these dogs.

Breur

# Compartmentalization

- Hyaline cartilage
  - Primary collagen is Type II
  - Collagen Type IX and XI also present
  - Collagen Type X
    - Immune cell
  - Primary proteoglycan is aggrecan
    - Others present throughout
    - COMP present locally
  - Compartments in matrix may sequester growth factors ( $\beta$  (TGF- $\beta$ ) and enzymes such as MMP's, alkaline phosphatase
  - Compartments may facilitate diffusion of nutrients, GF

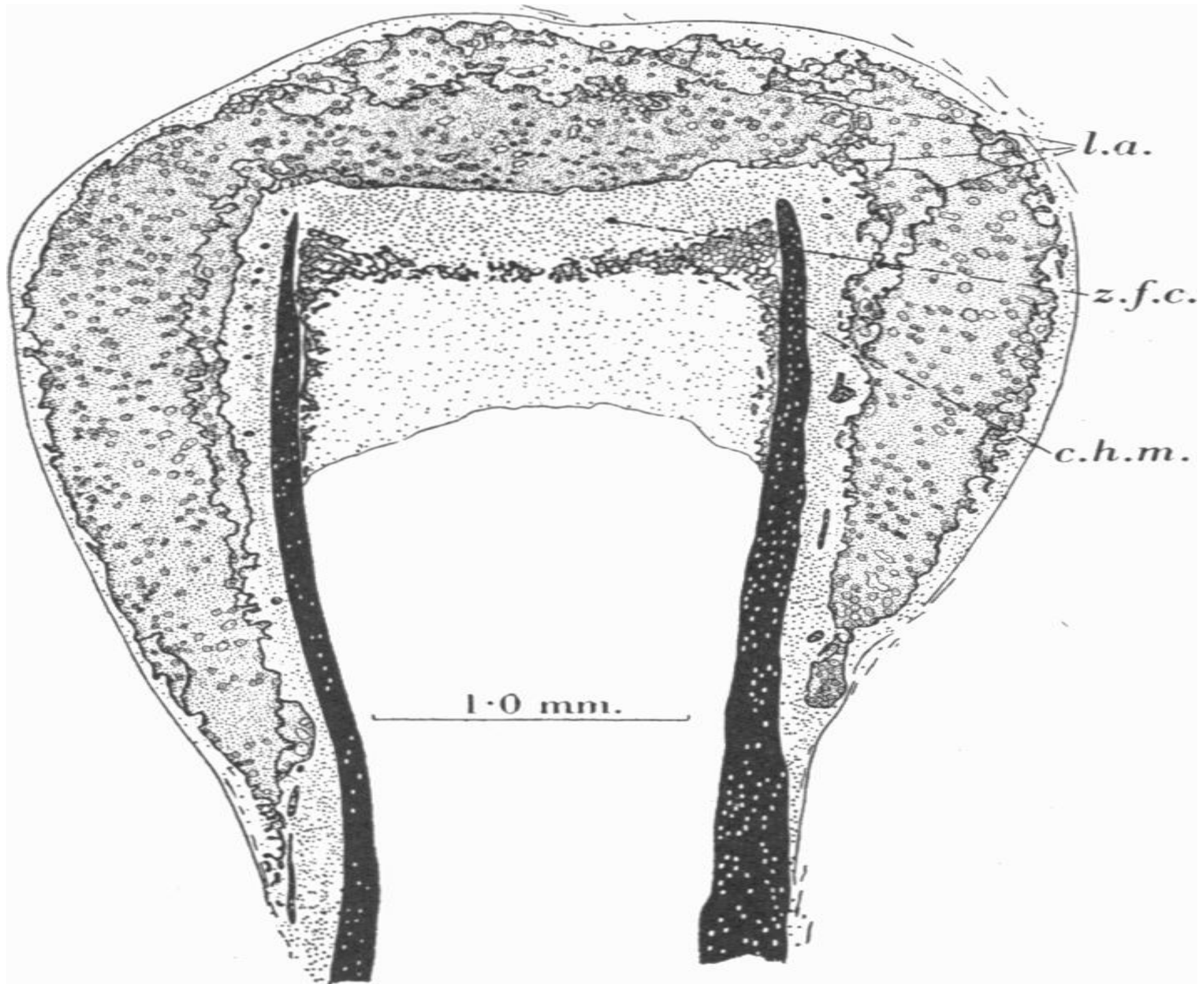
# Fish

- What's a fin?
  - Fin rays connected to endoskeleton by ligaments; grow by addition of segments
  - Not like digits; no counterpart in tetrapods
- Indeterminate growth thru EO
  - Usually secondary ossification center not present
  - Zones present, loose organization; no columns
- Wide variety of growth rates, extent of growth and final form of fin
- Teleost fish
  - Patterning
  - T-box gene— *tbx5*
  - Sox9—TF required for cartilage condensation, chondrocytic differentiation (as in mammals)



# Amphibians

- Many epiphyseal structures
- Different ratios of cartilage:enchondral bone:periosteal bone
  - Similar to fish for those primarily aquatic
  - Little enchondral bone and cylindrical periosteal bone in those primarily terrestrial
- Frogs
  - Malformations opportunity for study of regulation (every cloud has a silver lining)
- Unique epiphysis in *Rana spp.* (Haines, Next slide)
  - No columnar arrangement of chondrocytes
  - Division of chondrocytes perpendicular to long axis of bone
  - Hypertrophy not associated with mineralization or bone formation, no mechanism for translation of volume increase into growth
  - Periosteal ossification must drive elongation, growth cartilage adds to radial expansion, and EO is late (as animal gains weight)
  - Cartilage inserted into end of shaft, results in three regions:
    - Articular/lateral articular cartilage
    - Growth cartilage
    - Fibrous layer of periosteum (vascular)



# Reptiles

- Decoupled chondrification and ossification
- Natural selection acts on these two phases independently
- Postnatal development independent of prenatal patterning in iguanas (Maisano)
- Synchronization of PO vs EO varies
  - Alligators and Crocodiles—masses of hypertrophied cartilage isolated in bone marrow cavity, form cones, slowly replaced by bone
- Great variation in epiphyseal structure, indeterminate growth
  - Secondary ossification centers that really ossify
  - Centers that only calcify
  - No centers, epiphysis remains cartilaginous

# Birds

- Determinate
- Secondary ossification center lost?
  - Exists in proximal tibia only of most birds
  - Due to development of Cartilage canals? Air sac?
  - Dr. Sumida's note: these structures evolved early; not known at time of Haines work.
- Rate of elongation (post-hatch)
  - Adult size
  - Altricial versus precocial
  - The higher the growth rate, the greater the proportion of cartilage
  - Some birds exhibit growth rates of up to 6.0 mm (yes, that is millimeters!) per day in tibiotarsus
- Dinosaurs
  - Non-avian may never have had growth rates as high as modern birds
  - Selective pressure
  - Some dinosaurs did seem to grow rapidly, and attain gigantic size
    - 6 stages of growth noted in these animals:
      - Nestling (early and late) -- very high growth rate
      - Juvenile (early and late) --high growth rate (3 ½ m within 1-2 years)
      - Sub-adult-- growth slowing
      - Adult-- growth ceases, size 7 to 9 m at 6 to 8 years of age
      - Horton

# Birds vs Mammals

- Chick
  - Model organism
  - Regulatory pathways similar (pre/post natal)
- Chondrocytic cascade
  - Different emphasis between proliferation and hypertrophy
    - Proximal tibial growth plate of chick has long columns, cells are unorganized, metaphyseal vessels penetrate into HCZ
    - Numbers of cells, volume, cell cycle times correlate with growth
    - Rate of elongation greater in altricial hatchlings, due to greater volume of cartilage—the cost of more rapid bone elongation is reduced strength
  - Growth achieved by high cell turn-over
    - 6-55 cells/column/day which is more than 5X higher than in mammals
    - More cells produced per day by chick than rat
    - But, final hypertrophic cell volume is less (more efficient in rat)
    - Duckling
      - Distal tibiotarsus at 14 days of age grows 318 microns/day; cell volume is 2,710 cubic micrometers
  - Rat
    - Proximal tibia at 21 days of age 335 microns/day; cell volume 17,040 cubic micrometers

# Marsupials

- Differential growth

  - Opossum

    - Precocial development of forelimbs
    - Dissociation of growth rate
    - Forelimb develops faster at first, then hindlimb catches up
    - Similar in ducks (femur has faster growth rate)
    - Modular design/versatility

- Cartilage canals primitively absent

  - Role in formation of secondary ossification center
  - In rats and mice, cartilage canals have been lost



# Epigenetic factors

- Surgical correction of deformities, inequalities
  - Slowing growth more successful
  - Devices to control epiphyseal distraction
  - Stripping of periosteum (tension/increase of blood supply?)
- Loading
- Biomechanics
  - Range of skeletal form constrained by developmental processes; biophysical processes associated with tissue mechanical loading
- Lack of motion
  - Growth plate cartilage requires motion for elongation to occur
  - Cartilage—primary growth drive? (programmed early)
- Gymnasts
  - Bone maturation decreased
    - systemic changes
    - nutrition
  - Late acceleration of growth, final height higher than predicted



# Nutrition

- GH/IGF
  - programmed; possibly reprogrammed under stress
  - Regulated by nutrient/energy availability
- Large fetal life, growth rate more dependent on nutritional status than phenotype
  - Sampling environment, respond to future deficiency by programming for smaller size?
- Leptin
  - Feedback signal, GH/IGF and Thyroxine
  - Regulates energy homeostasis (adipose tissue storage)
- Catch-up Growth
  - Increased rate of growth (beyond normal limits) after period of inhibition
- 4-week old rats fasted for three days; elongation in proximal tibial growth plate 30% that of non-fasted littermates
- After 7 days of feeding, rates reached that of control group, and remained high for the next three weeks

Farnum

# Catching up.....

- Set point for length of individual bones
  - If growth interrupted, faster than normal elongation can be achieved under some circumstances
  - May not always be complete
    - Reprogramming?
- Altricial birds may respond to nutritional stress by slowing growth and maturation (fledge later)
  - Growth—increase in size
  - Maturation—changes in organ to bring to adult morphology, level of function
- Catch-up
  - NE Hypothesis:
  - Recognition of degree of mismatch (target to actual)
  - Growth adjusted in response (time-tally)
  - Growth Plate Hypothesis:
  - Senescence program (number of cell divisions by SC)
- Dog with non-treated femoral fracture as puppy
- Healed with shortening/widening of femur
- But, tibia compensatory overgrowth resulted in equal length of both limbs (joints at different levels)

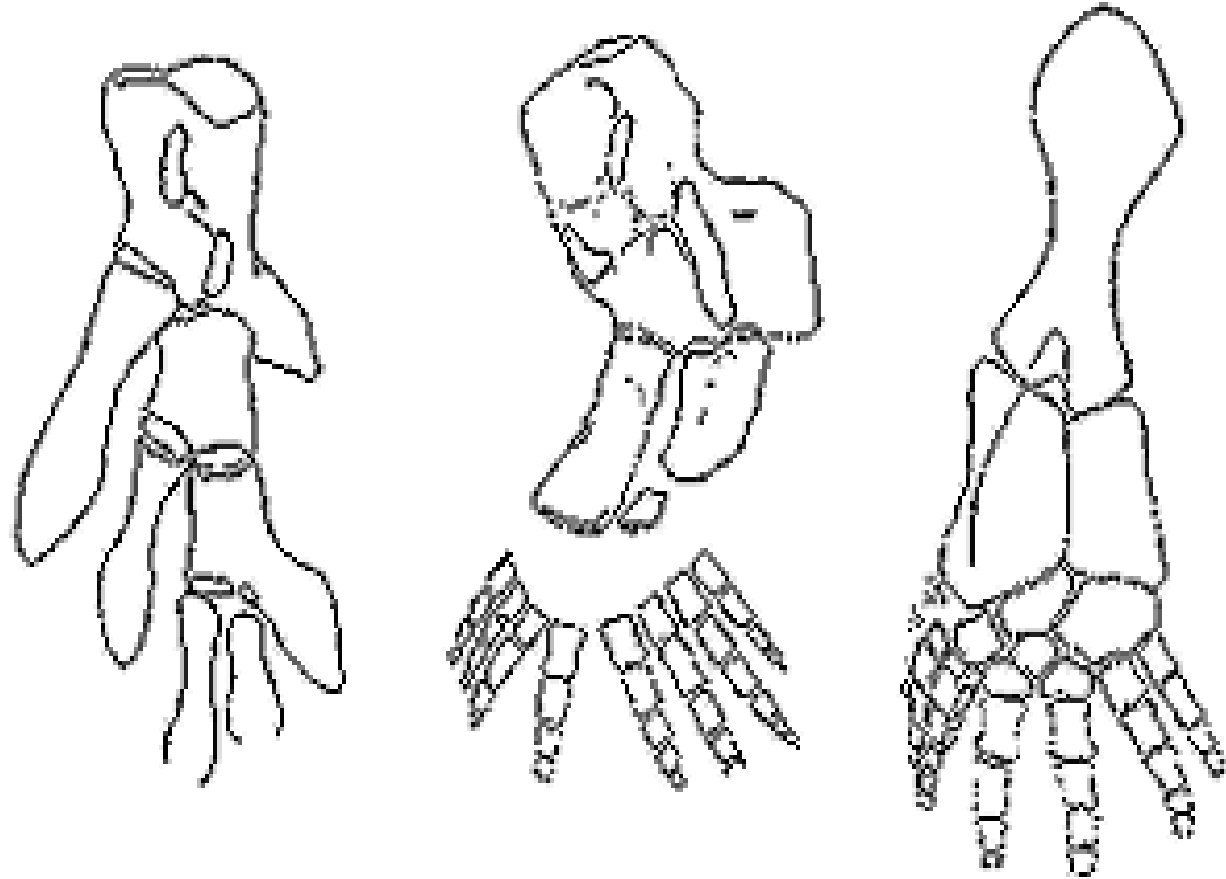
# Intrinsic factors

- Intrinsic program
  - Finite number SC + memory?
- Growth plate transplants
  - Skeletal length determined by factors inherent in each plate
    - Juvenile plates transplanted into older animals grow at juvenile rate (Kline)
- Growth plate closure
  - Usually rapid; timing similar across many species
  - If secondary center of ossification, proceeds from epiphyseal and diaphyseal side
  - Steroids required for growth/Estrogen for cessation
    - Estrogen from Testosterone via aromatase
- Directionality
  - If growth plate surgically rotated 180 degrees, original polarity remains
  - Bone with epiphyseal form grows in metaphyseal direction
  - Trabecular bone of epiphysis—woven appearance
  - Metaphyseal bone has longitudinal direction
  - intrinsic

# Morphological change

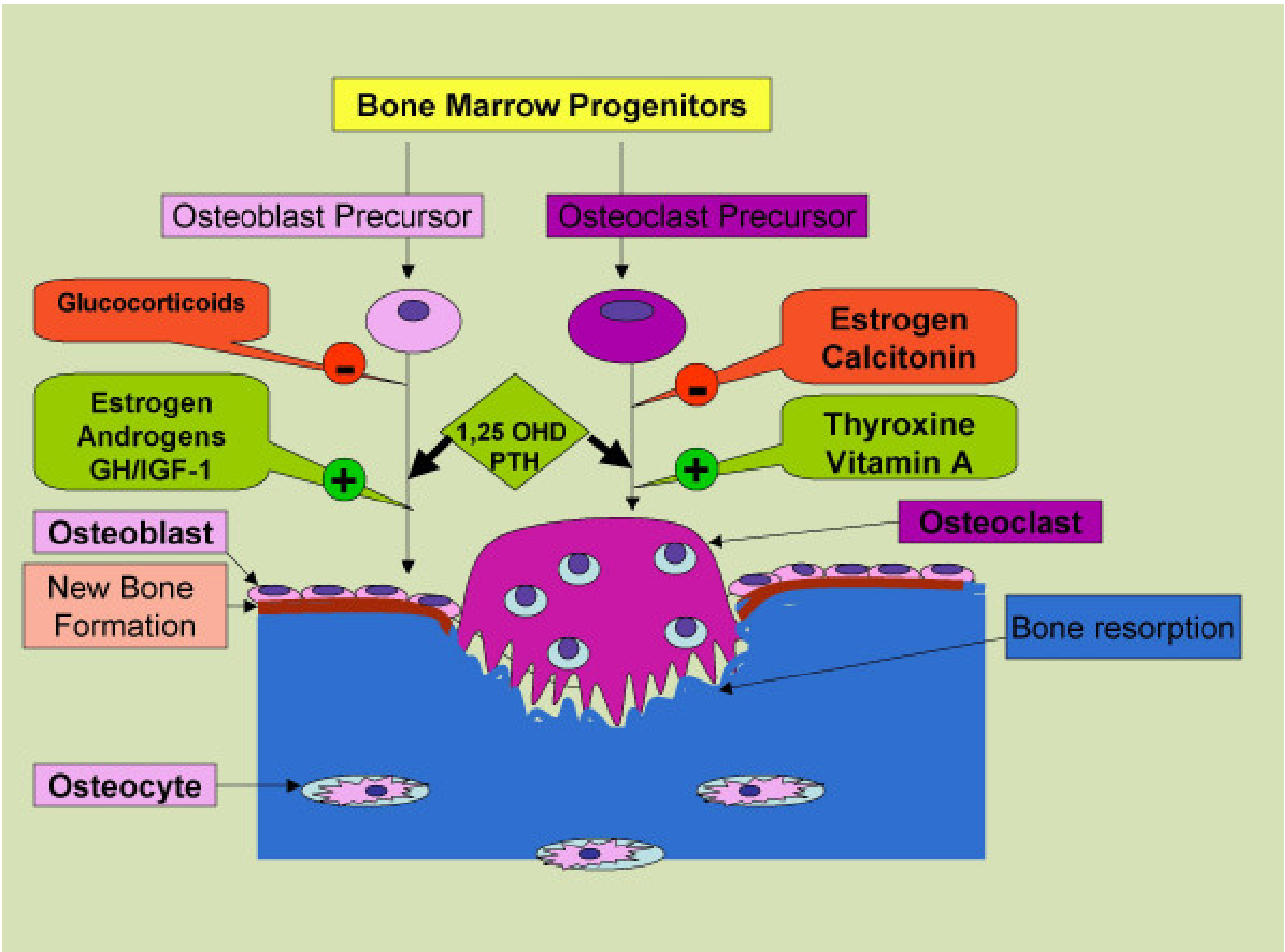
- Patterning genes
- Regulatory pathways
- Basic materials properties
- Modification of cartilage formation
- Modification of ossification
- Shifts in growth/duration
- Selection act at any level of organization: molecular, cell, tissue, organ
- Infinite possibilities
- “Challenge is to synthesize knowledge gained... into an ever more refined understanding...” Farnum

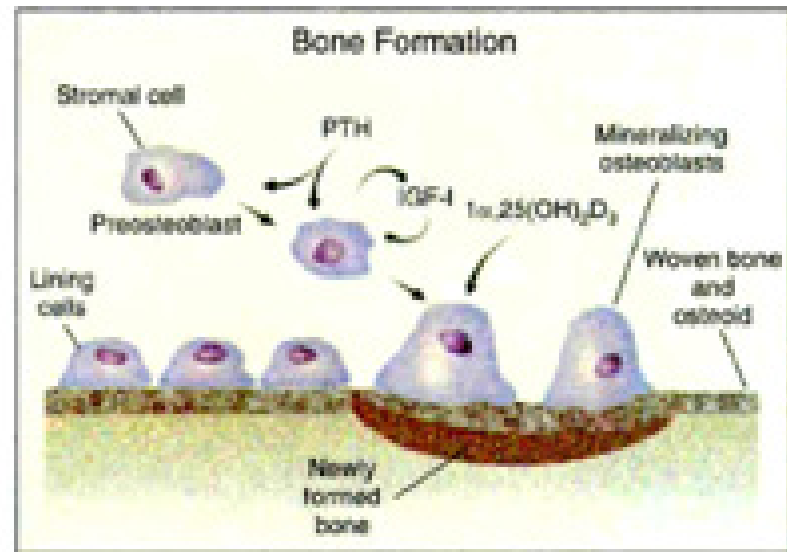
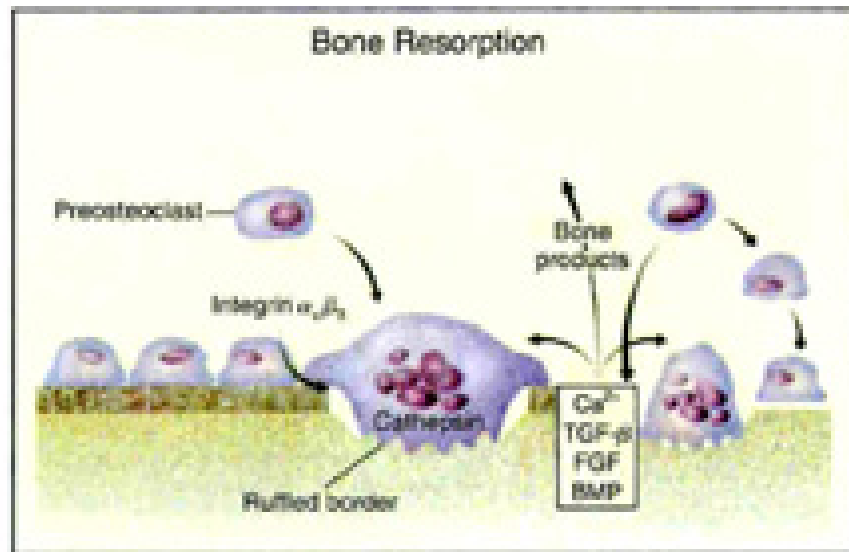
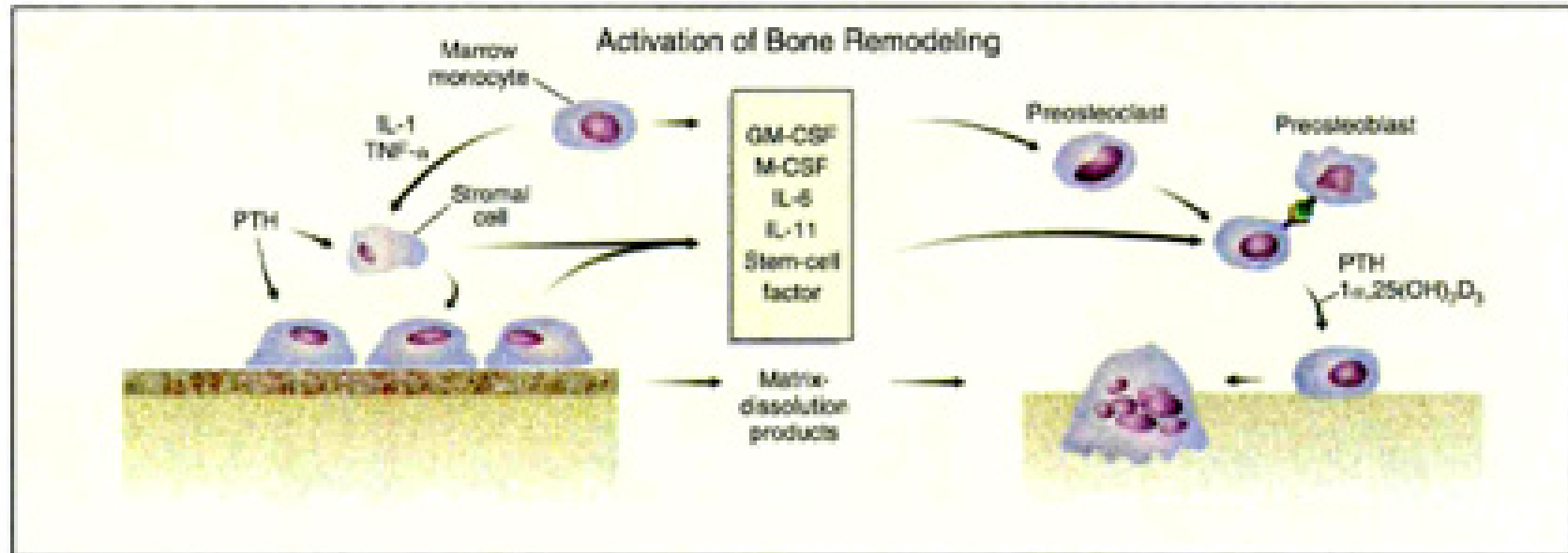
- The End
- Bye!



# Out-Takes

- They don't say Haines till I SAY they say Haines!  
– *Inspector 12*

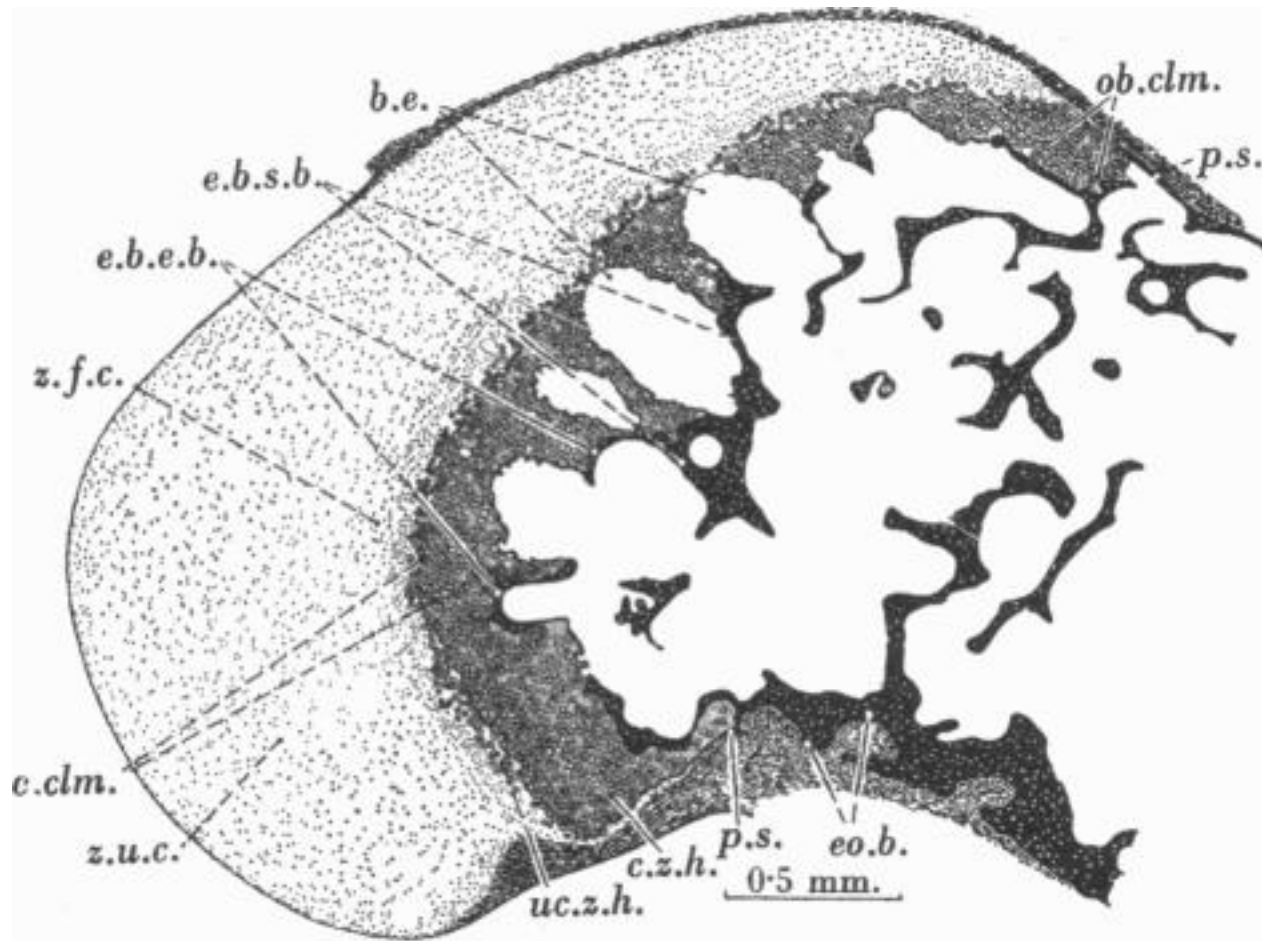


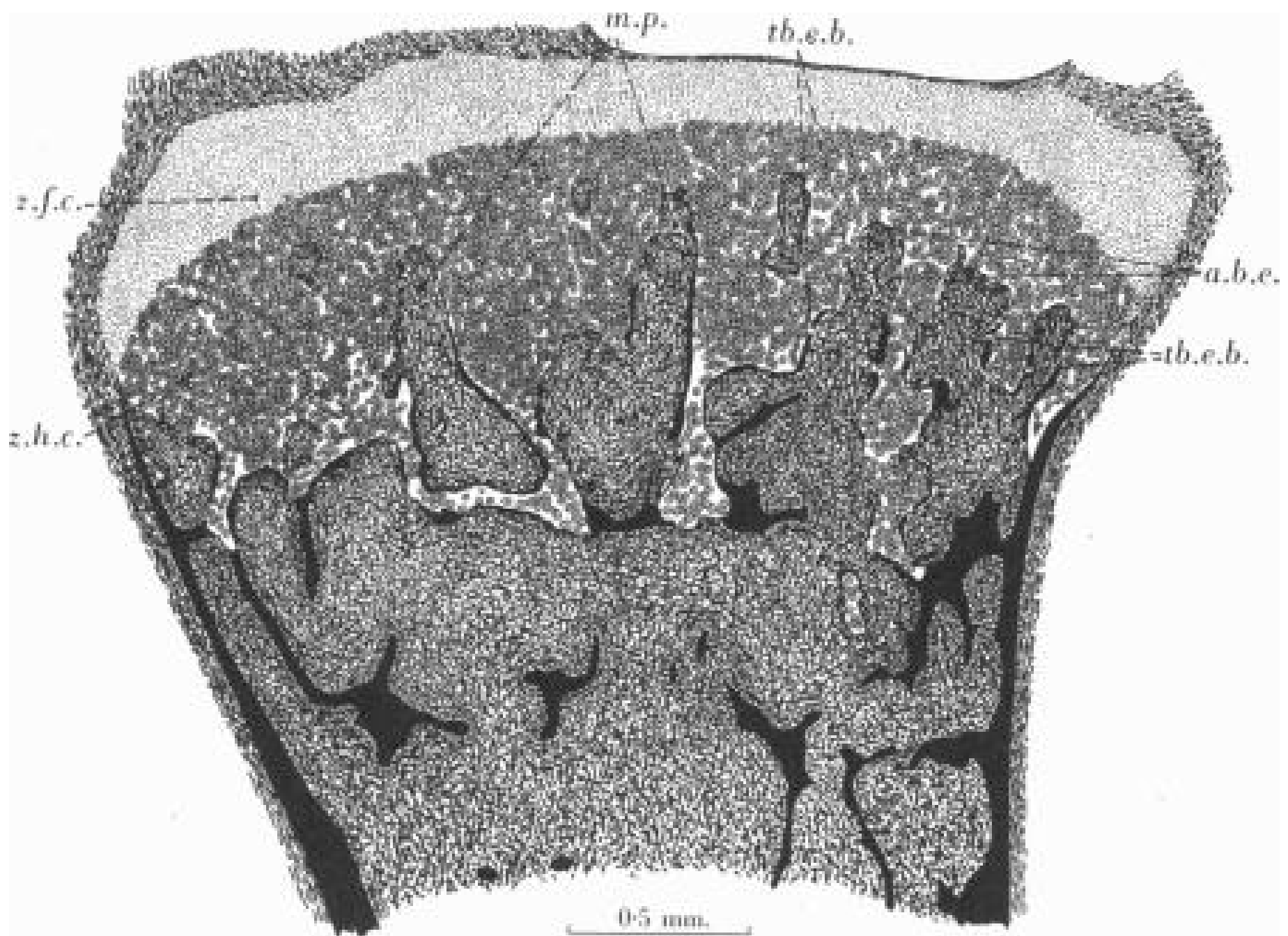


Hruska KA & Teitelbaum SL, 1995

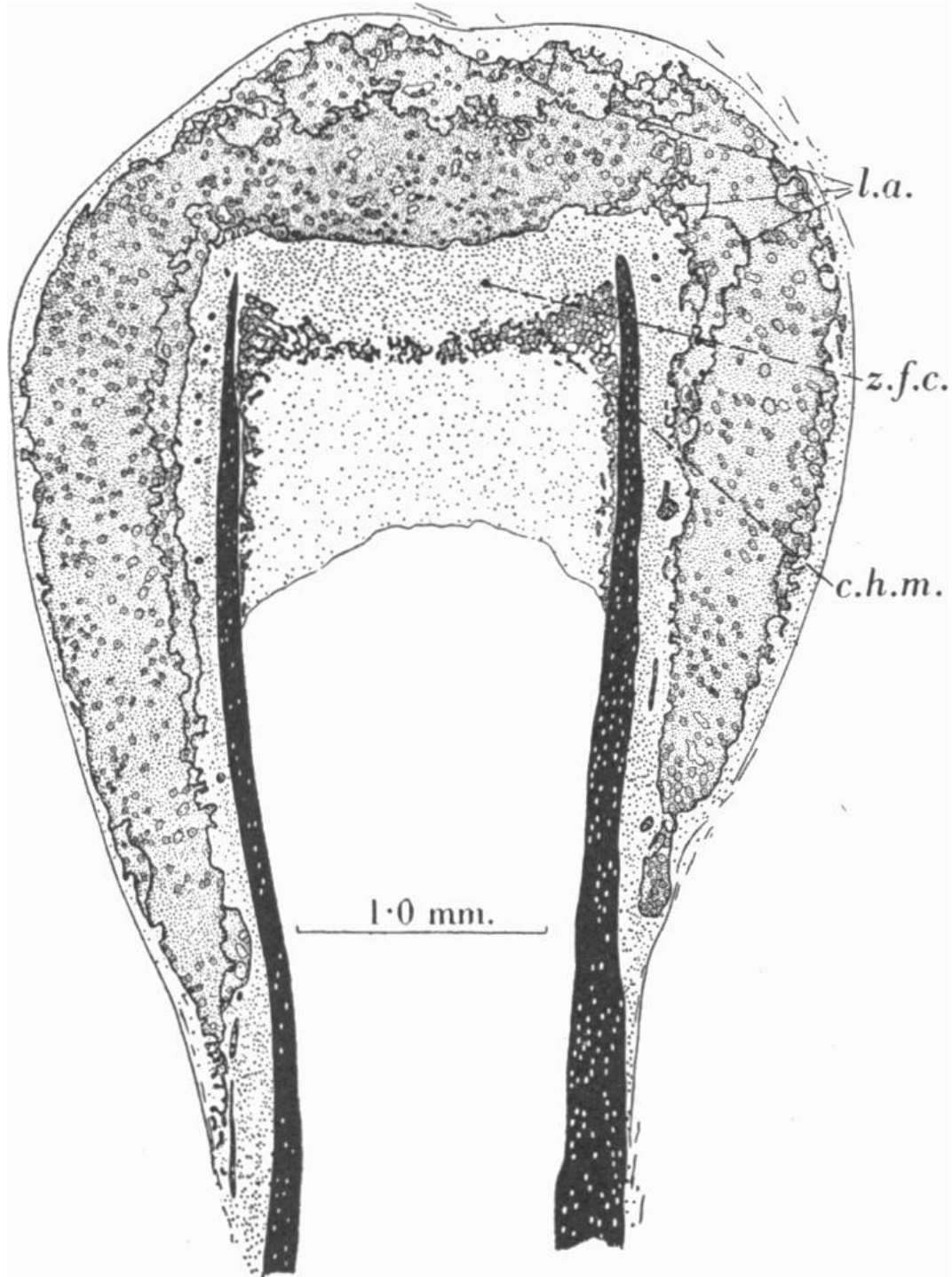


# Haines

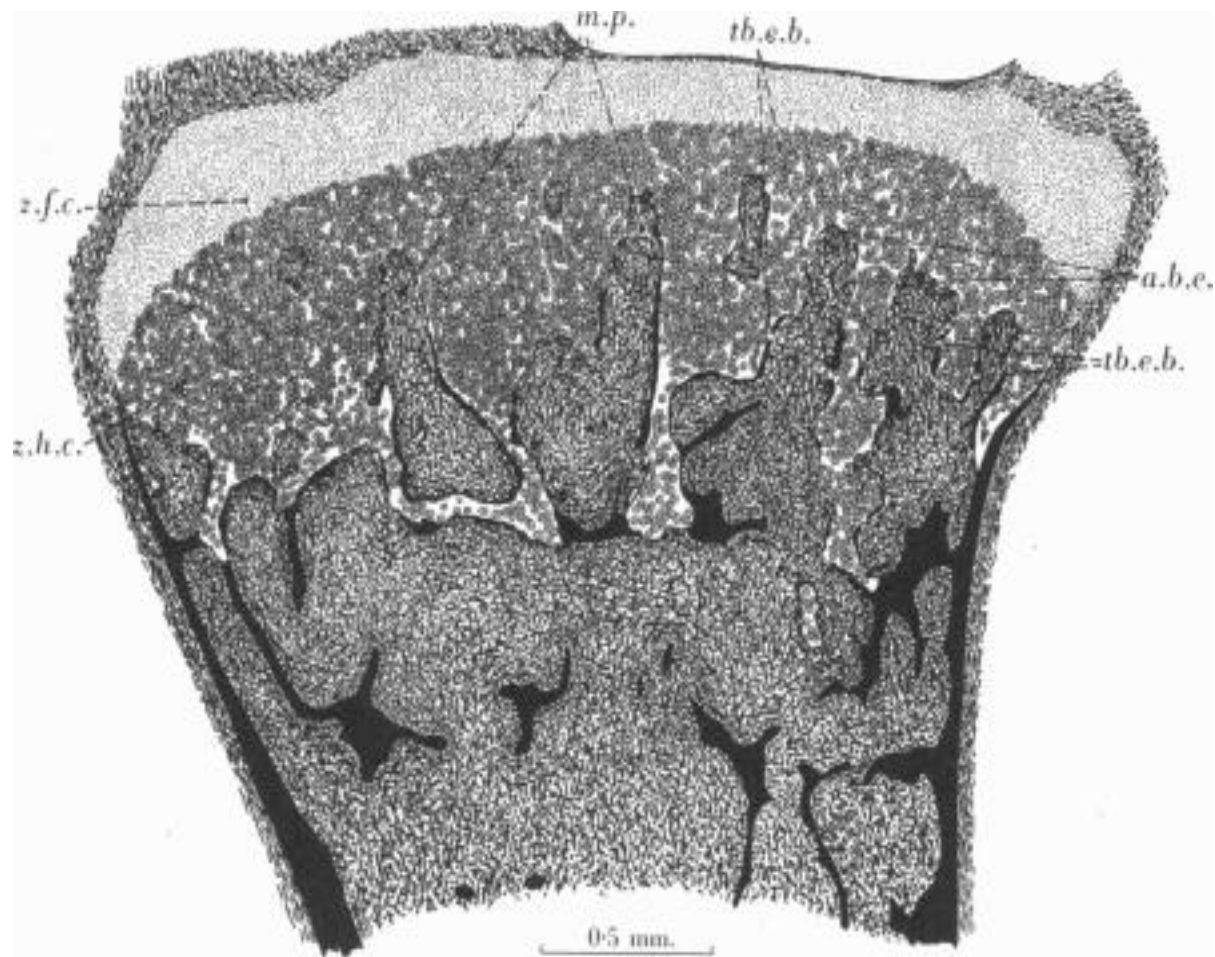




# Haines



# Haines



# Haines

