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Bone repair and stem cells

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Bones are an important component of vertebrates; they grow explosively in early life and maintain their strength throughout life. Bones also possess amazing capabilities to repair — the bone is like new without a scar after complete repair. In recent years, a substantial progress has been made in our understanding on mammalian bone stem cells. Mouse genetic models are powerful tools to understand the cell lineage, giving us better insights into stem cells that regulate bone growth, maintenance and repair. Recent findings about these stem cells raise new questions that require further investigations.

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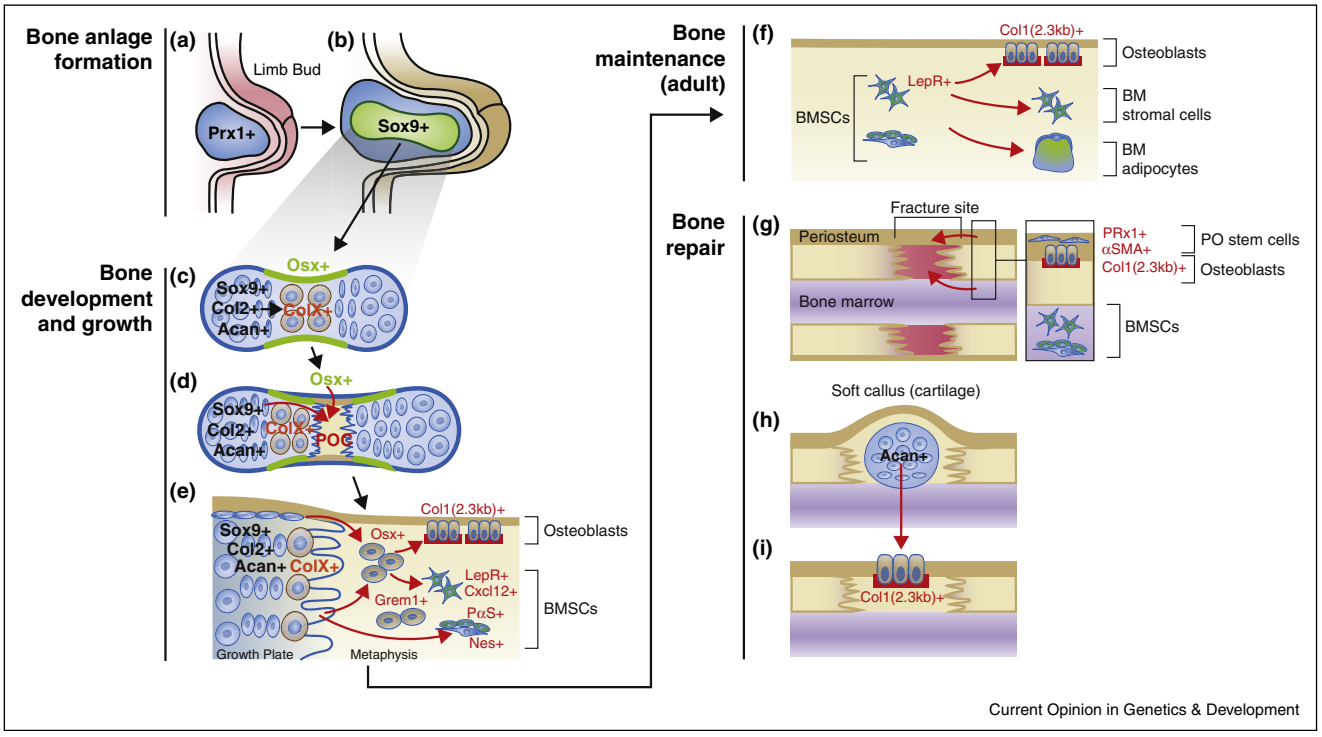
Introduction

One of the distinguishing features of vertebrates that explicitly discriminate them from invertebrates is their bones. Bones, with their strong and rigid structures due to mineralized matrix, provide protection for vital organs and act as levers whereby muscle contraction leads to movement of the body. Bones assume very different shapes in different part of the body, but they are formed through only two common mechanisms; intramembranous and endochondral bone formation. Intramembranous bone formation is a simple and straightforward process in which undifferentiated mesenchymal cells directly become osteoblasts that lay down the mineralized matrix. Intramembranous bones (or dermal bones) evolved earlier in the early fish, and comprise part of the skull in mammals. By contrast, endochondral bone formation is a complex process in which initial cartilage templates are replaced by bone. Most bones in mammals are formed through endochondral bone formation. Because of their primary function, bones are among the most commonly injured tissues of the body. Despite their inert appearance, bones continually turn over, replacing old bone with new and possess amazing capabilities to repair even after bone growth slows or stops. Not surprisingly, bone repair recapitulates the developmental sequence of the two modes of bone formation. Characterizing stem cells for bone growth, maintenance and repair has been largely hampered until recently due to technical and conceptual difficulties, including handling of mineralized hard tissues, complexity and plasticity of the bone cell development and lack of stage-specific markers or active promoters/enhancers identified in the early bone cells. Over the past few years, we have seen a substantial increase in our knowledge on this field. In this mini-review, we will discuss recent advances in the study of mammalian bone stem cells.

Stem cells for bone growth

Bone growth is substantial in early life, gradually slows down and eventually stops in adulthood. Therefore, stem cells for bone growth play active roles in early life while gradually slowing their action in later life, although the homeostatic turnover of adult bone continues throughout life. The relationship between stem cells needed for bone growth and stem cells needed for continuing renewal of bone during bone remodeling is uncertain. We will discuss endochondral bone formation of the limb as an example. The limb originates from the lateral plate mesoderm during embryogenesis. The transcription factor *Prrx1* is expressed in these mesodermal cells (Figure 1a). In fact, *Prrx1-cre*, in which *cre* recombinase is expressed under the direction of a 2.4 kb *Prrx1* promoter, marks essentially all limb mesenchymal cells in bones at a later stage, including osteoblasts, chondrocytes and stromal cells, but not muscle satellite cells [1]. Subsequently, a group of early mesenchymal cells within the limb bud condenses and determines the domain for the future cartilage and bone. The transcription factor *Sox9* is expressed in these mesenchymal cells (Figure 1b), and indeed is required for condensation [2]. These early mesenchymal cells develop into other mesenchymal cells in the cartilage and bone at a later stage, as *Sox9-cre* marks essentially all chondrocytes and osteoblasts, even though it is expressed only in mesenchymal precursors and in chondrocytes [3]. These fate-mapping experiments are consistent with the idea that stem cells for bone growth arise locally within the bone anlage. Sox9 directly binds to regulatory elements of cartilage-matrix genes, including those encoding *type II collagen* (*Col2*) and *aggrecan* (*Acan*). Cells within condensations start to express *Col2* and differentiate into chondrocytes, which then differentiate

Figure 1



Stem cells for bone growth, maintenance and repair. **(a)** *Limb bud formation*. Prrx1+ cells in the lateral plate mesoderm are the precursors for all other mesenchymal cells in bones at a later stage. **(b)** *Mesenchymal condensation*. Sox9+ cells are the precursors for all other chondrocytes and osteoblasts therefore determine the domain for the future bones. **(c)** *Cartilage formation*. Condensing mesenchymal cells soon differentiate into chondrocytes (Sox9+, Col2+ and Acan+) and establish the growth cartilage. These cells proliferate and further differentiate into hypertrophic chondrocytes (ColX+) at the center of the mold. The osteogenic perichondrium forms in its vicinity, where the first osteoblasts precursors (Osx+) appear. **(d)** *Ossification*. Blood vessels invade into the cartilage template attracted by angiogenic factors secreted by hypertrophic chondrocytes. Primary ossification center (POC) is formed when perichondrial and other mesenchymal cells from the cartilage move inside with blood vessels and proliferate. **(e)** *Growth*. The growth plate provides the primary engine for bone growth, and multiple types of mesenchymal cells are enlisted to form bones. A subset of growth plate cells (Sox9+, Col2+ and Acan+) continues to provide bone cells, some of which may pass through a ColX+ stage. Cells in the metaphysis, including Osx+ and/or Grem1+ cells are the precursors for osteoblasts and bone marrow stromal cells including BMSCs (LepR+, Cxcl12+, PαS+ and Nes+). **(f)** *Maintenance*. After bone growth stops, BMSCs (LepR+ and others) become the main source of osteoblasts, bone marrow stromal cells and adipocytes. **(g)** *Fracture repair*. When a complete fracture occurs, two distinct stem cell populations from the periosteum and the bone marrow respond to injuries. Periosteal stem cells (Prrx1+ and/or aSMA+) are the major source of chondrocytes in the soft callus, although BMSCs can contribute to chondrocytes to some extent. (H) *Soft callus*. Chondrocytes in the soft callus (Acan+) are the source of osteoblasts in the repaired bone. (I) Ossification of the fracture callus.

into hypertrophic chondrocytes expressing *type X collagen* (*ColX*) (Figure 1c). Around the same time that chondrocyte hypertrophy occurs, osteoblast precursors first appear in the surrounding region termed the perichondrium (Figure 1c). These perichondrial cells invade into the cartilage template along with blood vessels and become osteoblasts and stromal cells, establishing the primary ossification center (Figure 1d). *Runx2* and *osterix* (*Osx*), transcription factors essential for osteoblast differentiation [4–6], are expressed in a portion of the perichondrium near hypertrophic chondrocytes.

Lineage-tracing experiments using an *Osx-creERt* line demonstrate that fetal perichondrial Osx+ osteoblast precursors can indeed translocate into the ossification center [7]. As the bone anlage grows bigger, osteoblasts and stromal cells continue to proliferate as the primary ossification center expands. The perichondrial precursors that moved into the template can continue to proliferate only for a limited period, and eventually disappear [8•,9]. Interestingly, Osx+ cells marked in the early postnatal period, when the bone marrow is established, continue to generate stromal cells in the marrow for at least many months [8•,9]. Osx+ cells marked in adults do not have such capability [8•].

While the marrow space is being formed, the secondary ossification center develops within the epiphyseal cartilages remaining on both ends of the bone. The cartilage between the primary and secondary ossification centers is termed the growth plate, as it forms a disk with characteristics columns of chondrocytes (Figure 1e). The