

Putting the Brakes on Bone Growth

A fascinating story is emerging regarding the local control of linear bone growth. It has long been recognized that chondrocytes in skeletal growth plates progress through a complex differentiation process that involves proliferation and terminal differentiation (hypertrophy). Moreover, although a number of hormones and growth factors, most notably GH and IGF-1, are known to influence this progression, the local controls have remained poorly understood. Now, papers from 3 Boston research teams have defined a local negative feedback loop that serves as a brake on this process, controlling the rate of terminal chondrocyte differentiation (see Figure 1).

The feedback loop is simple; the proof of its existence was much more difficult. The loop has 2 major players: a signaling molecule called Indian hedgehog (Ihh) and parathyroid hormone-related protein (PTHrP). Ihh is 1 of at least 3 hedgehog proteins found in higher vertebrates that function as signal proteins, especially during early embryologic development. Hedgehog signals are thought to act through a receptor known as Patched (Ptc) and a transcription factor named Gli.

Through an elaborate series of experiments in developing chick limb buds and mouse embryos in which relevant genes were overexpressed and/or inactivated, the authors were able to determine the upstream and downstream relationships of loop components. First, they demonstrated that Ihh was produced by growth plate chondrocytes when they begin to terminally differentiate and that overexpression of Ihh suppressed terminal differentiation. Next, they showed that Ptc receptor and Gli transcription factor were expressed in perichondrial cells around the periphery of the growth plate.

Related experiments showed that PTHrP is synthesized by periarticular perichondrial cells and that PTHrP receptor, which is also a receptor for PTH, is expressed by proliferating chondrocytes just prior to terminal differentiation. Genetic inactivation of the receptor was associated with accelerated

chondrocyte terminal differentiation. Finally, the loop was closed when Ihh suppression of terminal differentiation was shown to depend on PTHrP.

The proposed model is shown in Figure 1. Briefly, as growth plate chondrocytes decide to terminally differentiate, they express high levels of PTHrP receptor. Once committed to this fate, they transiently express Ihh, which acts on the adjacent perichondrial cells through Ptc and Gli to directly or indirectly cause periarticular perichondrial cells to secrete PTHrP. PTHrP signals back to proliferating chondrocytes expressing PTHrP receptors, preventing them from progressing down the terminal differentiation pathway. Thus, the loop functions as a brake on terminal differentiation, essentially controlling the number of cells terminally differentiating at any given time.

Roush W. *Science*; 1996;273:579.

Vortkamp A, et al. *Science* 1996;273:613-622.

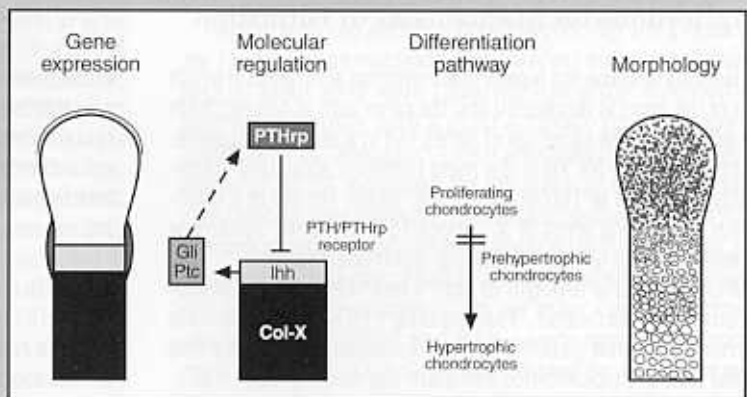
Lanske B, et al. *Science* 1996;273:663-666.

Editor's comment: This work provides a new context in which to consider control of bone growth. The greatest uncertainty is how Ihh signals to periarticular perichondrial cells that secrete PTHrP. Nevertheless, that PTHrP is required for Ihh inhibition of chondrocyte terminal differentiation is hard to dispute. Given the long distances in this model that PTHrP must diffuse through cartilage matrix, a recognized barrier to diffusion of many molecules—especially in larger bones such as those in humans—it is difficult to imagine how this feedback loop would be responsible for the fine-tuning of subtle events in the growth plate. As the authors imply, perhaps this loop is one of several locally acting mechanisms that control skeletal development and growth.

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Figure 1
Proposed Regulation of Cartilage Differentiation During Bone Growth

Ordinarily, growth plate chondrocytes become hypertrophic chondrocytes, briefly passing through a prehypertrophic stage during which they sequentially express the PTH/PTHrP receptor and Ihh genes. The Ihh signal is transmitted to the perichondrium, where it elicits expression of another set of genes, Gli and Ptc, which leads to expression of PTHrP in the periarticular perichondrium. PTHrP then signals back to its receptor in the prehypertrophic cells to block progression of more cells down the hypertrophic chondrocyte pathway, ie, it closes the negative feedback loop. As chondrocytes fully hypertrophy, Ihh expression ceases, which releases the "brake" imposed by the negative loop, allowing more cells to enter the hypertrophic pathway.



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