

could alter growth factor signaling within the growth plate, as proteoglycans are thought to influence the mobility and local concentrations of growth factors

in cartilage and possibly their presentation to transmembrane receptors.

William A. Horton, MD

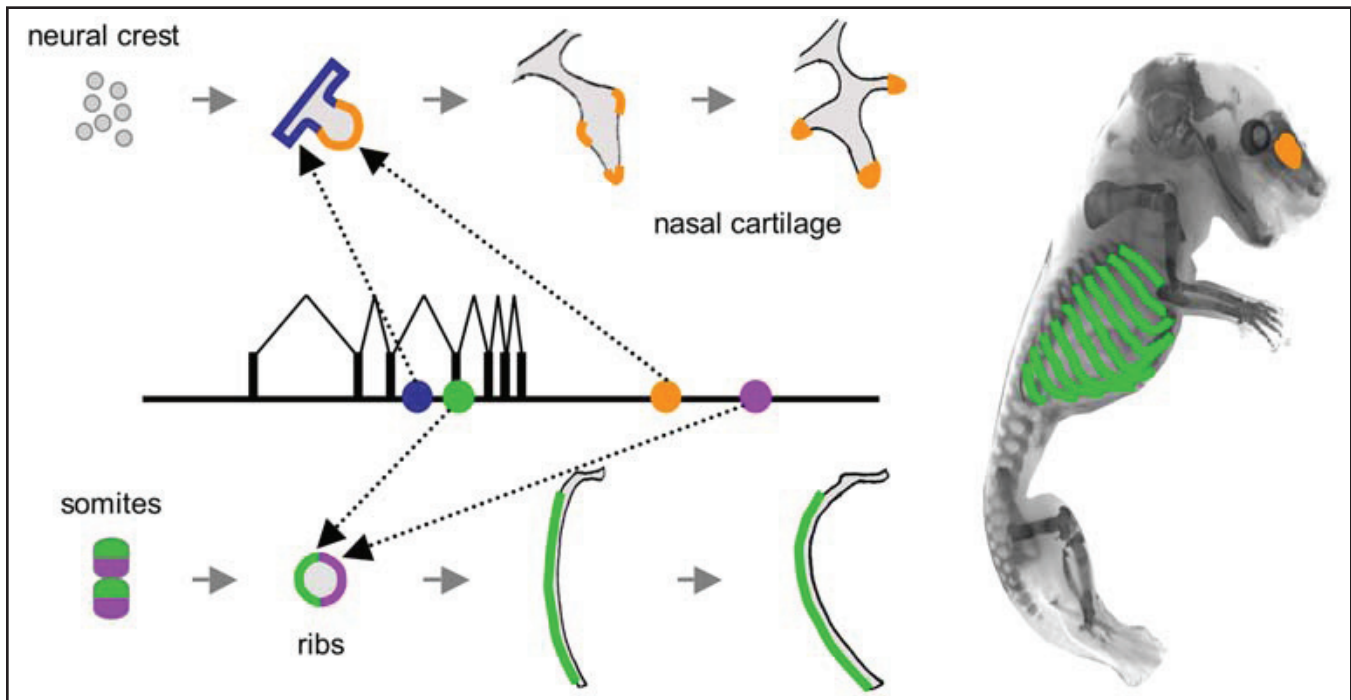
Anatomy-Specific Enhancers of BMP Genes Fine-Tune Size and Shape of Individual Bones

As the skeleton grows, constituent cartilage and bone tissues are formed into a remarkable range of sizes and shapes. Although the blueprints that sculpt individual bones must be encoded in the genome, little is known about how this occurs. A group headed by David Kingsley at Stanford has recently provided novel insight into how the anatomy of individual bones is regulated. Their story begins with the generally accepted concept that bone shapes are determined by differential growth and erosion along the surfaces of bones. For instance, preferential deposition and erosion on opposite surfaces of a bone would generate lateral displacement or curvature of the bone such as a rib. Localized regions of deposition and erosion would shape ridges, foramina, and other surface structures.

The group focused their attention on the BMP5 gene because it is surrounded by large genomic regions containing regulatory elements required for normal developmental regulation and on rib development because BMP5 is expressed in the perichondrium surrounding

ribs and ribs are suitable for detecting differential growth and erosion. The approach was to generate transgenic mouse embryos harboring both a *lacZ* reporter gene and genomic DNA corresponding to different regions of the BMP5 locus including surrounding genomic DNA. β -galactosidase staining of late-stage transgenic embryos revealed specifically where the regulatory regions, ie, presumed enhancers, were active.

The details of the experiments are beyond the scope of this abstract. However, a regulatory element within the coding region of the gene was found to drive expression of BMP5 in the perichondrium adjacent to the lateral aspect of the ribs, whereas regulatory sequences 100 kb 3' to the coding region drove expression in the perichondrium of the medial aspect of the ribs. A number of confirmatory experiments was done, all of which suggested that BMP5 expression in different domains of the rib perichondrium is controlled by distinct regulatory elements in or near the BMP5 locus. In other words, anatomy-specific enhancers in BMP genes may provide



Discrete enhancers control growth in distinct anatomical domains of developing bones. Multiple anatomy-specific enhancers (filled circles) are spread across the *Bmp5* locus. In ribs, 2 enhancers (green and purple circles) may respond to lineage domains established in somites to control growth on opposing sides of the ribs. Local growth on the lateral edge of rib surfaces promotes rib curvature and expansion of the thoracic cavity. Nasal cartilages form from cranial neural crest. Two enhancers (blue and orange circles) in the *Bmp5* gene are expressed in different highly restricted locations, leading to characteristic branching patterns of the nasal turbinates.

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a genomic mechanism for independent developmental control of local growth of individual bones.

During these studies, the investigators also discovered 2 regulatory elements that controlled expression of BMP5 in nasal cartilage. These elements were distinct from those controlling BMP expression in ribs but like them mapped to locations within and 3' from the coding region of the gene as shown in the Figure. The authors suggested that the proposed mechanism may not be limited to regulation of BMP5 but common to other developmentally regulated genes that are involved in fine-tuning morphogenesis.

Guenther C, Pathalena-Filho L, Kingsley DM. Shaping skeletal growth by modular regulatory elements in the Bmp5 gene. *PLoS Genetics*. 2008;4:1-13.

Editor's Comment: This investigation provides novel insight into the fine-tuning of skeletal development. It is interesting to speculate how subtle radiographic findings that allow experts to distinguish between similar bone dysplasias might reflect disturbances in these regulatory mechanisms.

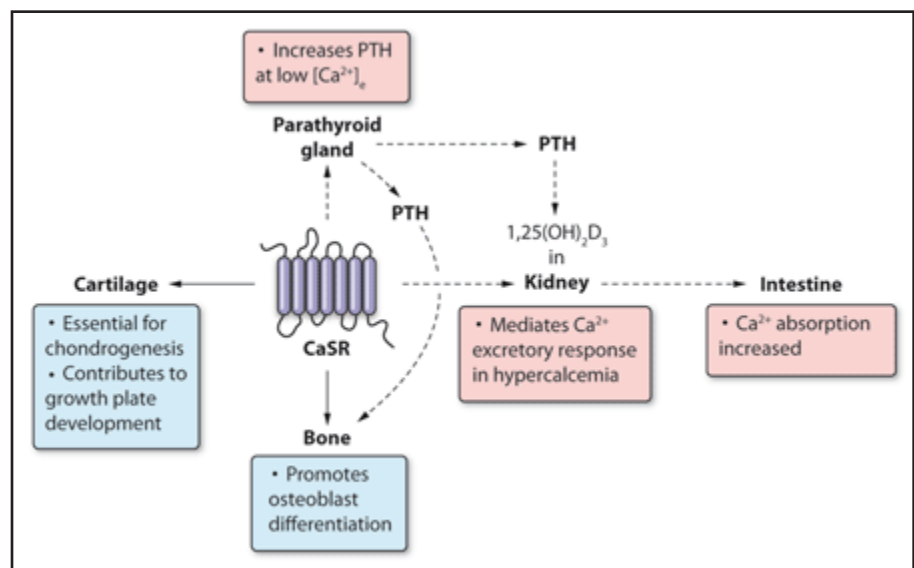
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Extracellular Calcium-Sensing Receptor: Modulator of Skeletal Development

The roles of the calcium-sensing receptor (CaSR) as a negative regulator of parathyroid hormone (PTH) synthesis and secretion and as an inhibitor of calcium reabsorption by the renal tubule are well documented; its functions in chondrocytes, osteoblasts, and osteoclasts have been less completely documented. In part, this has been due to inability to generate mice in which *Casr* has been ablated specifically in cartilage and bone cells and to the expression in these cells of an alternatively spliced, functionally active isoform of the CaSR.¹ *Casr* is expressed in differentiating osteoclasts, chondrocytes, and osteoblasts. Generalized ablation of *Casr* in mice results in a rachitic phenotype (increased width of the zone of hypertrophic chondrocytes, depressed and disordered calcification of the cartilage growth plate, and decreased rate of cartilage mineralization).²

The present investigators have developed strains of mice in which exon 7 of *Casr* (encoding the 7 transmembrane and 4 intracellular loops of the receptor protein) has been specifically "knocked-out" in parathyroid cells (PTC), growth plate chondrocytes (GPC), and osteoblasts (OB) rendering the *Casr* functionally inactive. Homozygous loss of *Casr* in PTC resulted in impaired growth and death within 2 weeks after birth. As anticipated, these mice had increased expression of *Pth* in their PTC. The skeletons of these mice had abundant matrix but were markedly undermineralized, and multiple fractures were present. There was substantially decreased expression of *Casr* in bone cells

and delayed OB differentiation. In mice in which *Casr* was specifically ablated in OB, the phenotype of growth retardation, skeletal undermineralization with increased osteoid formation, multiple fractures, and death by 3 weeks of age was observed. OB differentiation was severely impaired as was OB expression of *Igf1*. The rate of apoptosis of OBs was accelerated. The homozygous loss



Classic Ca^{2+} homeostasis (dashed arrows) and novel developmental functions (arrows) of CaSR have been revealed by cell type-specific null mutations of *Casr* in the mouse. CaSR is found in bone, kidney, and gut, which are the three main Ca^{2+} -mobilizing organs. The normal homeostatic signaling pathways between these organs and the parathyroid gland have been detailed previously.⁵ The functions performed by CaSR in each organ are outlined in boxes. To maintain normal Ca^{2+} homeostasis, CaSR in parathyroid cells (PTCs) senses alterations in $[Ca^{2+}]_e$. The release of parathyroid hormone (PTH) enables bone and kidney to respond in a manner to normalize $[Ca^{2+}]_e$, through the activation of key responses in kidney [production of $1,25(OH)_2D_3$ and reabsorption of Ca^{2+}], intestine [Ca^{2+} absorption through the increased abundance of $1,25(OH)_2D_3$], and bone matrix resorption through PTH (not shown). The direct role of CaSR in the intestine is questionable because an intestine-specific knockout of *Casr* has not been performed. Targeted knockout of *Casr* through the crossing of *Casr* floxed mice with mice expressing *Cre* under the control of tissue-specific promoters has identified novel functions for CaSR in skeletal development. Ablation of *Casr* in the parathyroid gland resulted in the expected phenotypes that occur in patients with inactivating mutations in *Casr*, such as hyperparathyroidism and hypercalcemia. Deletion of *Casr* in chondrocytes demonstrated a requirement for CaSR in early skeletal development, whereas a role for CaSR in promoting bone cell differentiation was determined by deletion of *Casr* in cells of the osteoblast lineage. Reprinted with permission Brown EM, Lian JB. *Sci Signal*. 2008;1; pe40. Copyright © AAAS 2008. All rights reserved.