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Targeted overexpression of parathyroid hormone-related peptide in chondrocytes causes chondrodysplasia and delayed endochondral bone formation

(developmental regulation/programmed differentiation/chondrodystrophy)

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ABSTRACT **Parathyroid hormone-related peptide (PTHrP) was initially identified as a product of malignant tumors that mediates paraneoplastic hypercalcemia. It is now known that the parathyroid hormone (PTH) and PTHrP genes are evolutionarily related and that the products of these two genes share a common receptor, the PTH/PTHrP receptor. PTHrP and the PTH/PTHrP receptor are widely expressed in both adult and fetal tissues, and recent gene-targeting and disruption experiments have implicated PTHrP as a developmental regulatory molecule. Apparent PTHrP functions include the regulation of endochondral bone development, of hair follicle formation, and of branching morphogenesis in the breast. Herein, we report that overexpression of PTHrP in chondrocytes using the mouse type II collagen promoter induces a novel form of chondrodysplasia characterized by short-limbed dwarfism and a delay in endochondral ossification. This features a delay in chondrocyte differentiation and in bone collar formation and is sufficiently marked that the mice are born with a cartilaginous endochondral skeleton. In addition to the delay, chondrocytes in the transgenic mice initially become hypertrophic at the periphery of the developing long bones rather than in the middle, leading to a seeming reversal in the pattern of chondrocyte differentiation and ossification. By 7 weeks, the delays in chondrocyte differentiation and ossification have largely corrected, leaving foreshortened and misshapen but histologically near-normal bones. These findings confirm a role for PTHrP as an inhibitor of the program of chondrocyte differentiation. PTHrP may function in this regard to maintain the stepwise differentiation of chondrocytes that initiates endochondral ossification in the midsection of endochondral bones early in development and that also permits linear growth at the growth plate later in development.**

Parathyroid hormone-related peptide (PTHrP) was initially identified as the tumor product that is responsible for most instances of humoral hypercalcemia of malignancy. In this circumstance, tumors elaborate PTHrP into the systemic circulation in sufficient quantity to cross-react with classical parathyroid hormone (PTH) receptors in bone and kidney, an interaction that provided the initial biochemical clues to the existence of PTHrP and also served as the basis for bioassays that were used in its purification (1–3). PTHrP does not normally appear to circulate, however, so that such effects are of more pathophysiological than physiological relevance (3).

The PTH and PTHrP genes arose on the basis of an ancient duplication event, and they share a similar genomic organization as well as a short segment of homologous amino-terminal peptide sequence that, in both peptides, seems to interact with a single G-protein-coupled receptor, termed the PTH/PTHrP receptor (1–4). This sharing of a common receptor is unusual because the functions of PTH and PTHrP seem to be entirely unrelated. PTH is a product exclusively of the parathyroid chief cell, and it is charged with maintaining systemic calcium and phosphorous homeostasis. In contrast, PTHrP is expressed in a remarkable variety of normal adult and fetal tissues (3), findings that have been taken as evidence for autocrine/paracrine function. The PTH/PTHrP receptor has been found to be also widely expressed, frequently in cells immediately adjacent to PTHrP-producing cells, another point favoring paracrine function (5). Thus, the specificity of signaling of this small peptide family appears to be entirely a function of the spatial and temporal expression of the two ligands and their shared receptor.

The study of putative local effects of PTHrP in such a wide variety of tissues has proven to be a challenge to those interested in function. However, the power of modern gene targeting and disruption strategies has recently provided remarkable functional insight, in that each of a number of such experiments has produced a developmental phenotype, the net result being clear evidence that PTHrP is a developmental regulatory molecule. For example, targeted overexpression of PTHrP to skin and breast has resulted in a delay/failure of developmental programs in these tissues (6, 7), while mice with a disrupted PTHrP gene die at birth with a form of chondrodysplasia that seems to be based on an accelerated program of chondrocyte differentiation (8). The present manuscript describes an experiment in which PTHrP overexpression was targeted to chondrocytes using the type II collagen promoter. This targeting results in a profound delay in chondrocyte differentiation as well as a pattern of endochondral bone formation that seems to be essentially reversed compared with the normal pattern.

MATERIALS AND METHODS

Generation and Identification of Transgenic Mice. A 568-bp human PTHrP cDNA fragment encoding the 1–141 isoform (6) was inserted into a 6.5-kb segment of the mouse procollagen α1 type II (col II) promoter region (9–13) (obtained from S. Garofalo, Shriners Hospital, Portland, OR, and B. de Crombrugghe, M. D. Anderson Cancer Center, Houston, TX) at a site within the first intron (ref. 14 and Fig. 1). A consensus

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Abbreviations: col II, procollagen a1(II); JMC, Jansen-type metaphyseal chondrodysplasia; PTH, parathyroid hormone; PTHrP, parathyroid hormone-related peptide.

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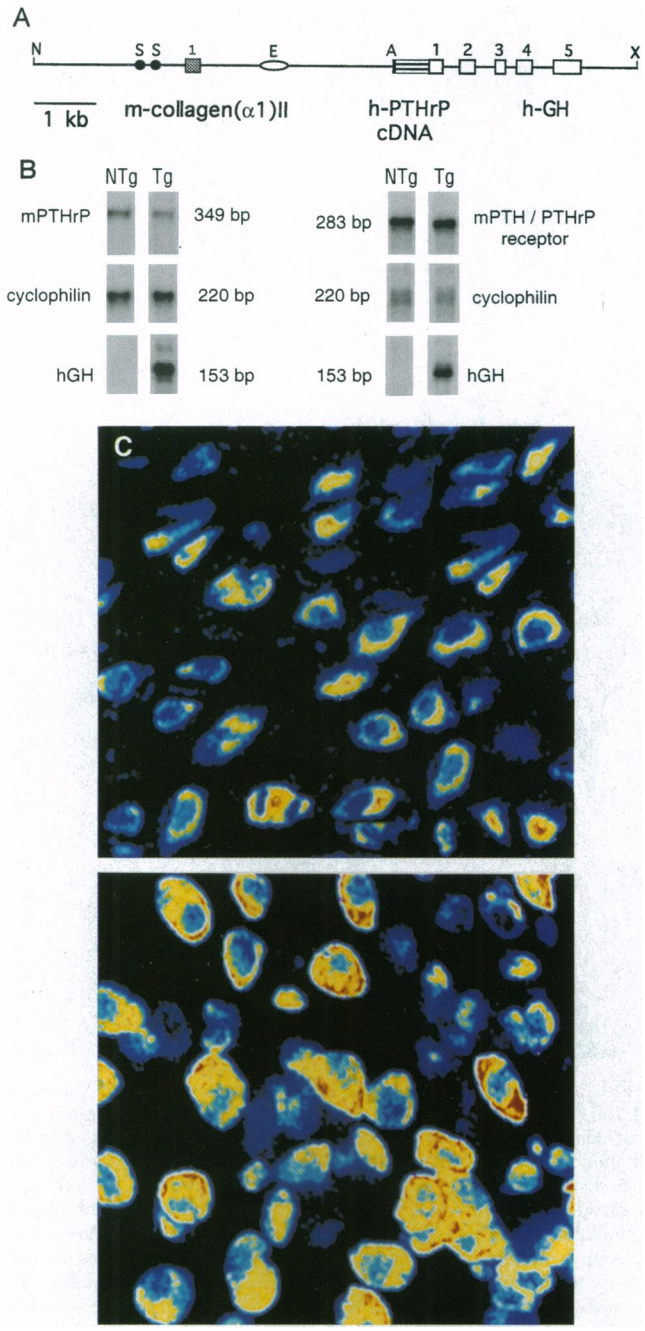

FIG. 1. Construction and expression of the transgene. (*A*) Map of the col II–PTHrP transgene. The construct was designed to encompass the essential regulatory elements present in the mouse α 1 type II procollagen gene promoter region, including a chondrocyte-specific enhancer (E) in intron 1 and a pair of transcriptional silencers (S) that suppress gene expression in cell-types other than chondrocytes (10–13). This gene segment has been shown to recapitulate the spatial and temporal expression pattern of the endogenous procollagen gene (14). This gene is expressed at 9.5 days post coitus in chondroprogentior cells, in the inner layer of the perichondrium, and at high levels in proliferative and prehypertrophic chondrocytes during all stages of cartilage development (15–17). It is also expressed transiently during development in the heart and epidermis (15, 16). Exons are boxed, with shading for mouse procollagen sequences, stripes for the human PTHrP cDNA, and open boxes for human growth hormone sequences. A denotes the splice acceptor. N, *Not*I; X, *Xba*I. Forward and reverse primers for growth hormone were CTACGGGCTGCTCTACTGCTTCAGG and GGCACTGGAGTGGCAACTTCCAAGG and for GAPDH were CGTGGAGTCTACTGGTGTCTTCACC and GATGGCATGGACTGTGGTCATGAGC. (*B*) RNase protection analysis of total RNA prepared from limbs of 1-week-old transgenic mice (Tg) and nontransgenic littermates (NTg). To examine transgene expression, 30 μg of total RNA was analyzed for human growth hormone expression (hGH). Expression of endogenous murine PTHrP (mPTHrP) and the murine PTH/PTHrP receptor was also examined in separate assays. Cyclophilin was included as a loading control. The size of the protected fragments is indicated beside each panel. (*C*) Confocal immunohistochemical staining for PTHrP. A segment of the femoral growth plate of a 5-day-old nontransgenic mouse (*Upper*) and an equivalent cell population in the femur of a transgenic littermate (*Lower*) are shown.

splice acceptor (courtesy of P. Soriano, Fred Hutchinson Cancer Research Center, Seattle) was included in the col II construct at the 5′ end of the insertion point to create an artificial exon and thus circumvent alternative splicing (14). Also, the initiation codon in exon 1 of the procollagen gene (14) was inactivated to allow translation to start within the cDNA sequences. A 2.2-kb segment of the human growth hormone gene was added downstream of the cDNA to provide termination/polyadenylylation signals and to increase expression efficiency; the growth hormone coding sequences are not translated (6). The assembled transgene was then microinjected into fertilized C57BL/6 × SJL F_2 oocytes, and resultant col II–PTHrP transgenic mice were identified by PCR amplification of a 171-bp sequence within exon 5 of the growth hormone portion of the transgene. The integrity of genomic DNA was assessed by coamplification of a 259-bp segment of the endogenous murine glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene.

Analysis of Transgene Expression. For the preparation of total cellular RNA, bones were rapidly dissected and immediately frozen in liquid nitrogen prior to being crushed and then solubilized in Trizol reagent (GIBCO/BRL). RNase protection assays were performed as described (6) using RNA probes corresponding to the following fragments: (*i*) a 153-bp *Bgl*II–*Pvu*II segment from exon 5 of the human growth hormone gene, (*ii*) a 349-bp *Avr*II–*Pvu*II segment from the principal coding exon of the murine PTHrP gene, (*iii*) a 283-bp *Sau*3a–*Pvu*II segment of the murine PTH/PTHrP receptor cDNA, and (*iv*) a 220-bp *Sau*3a–*Sau*3a exonic segment of the mouse cyclophilin gene (6).

Histology and Immunohistochemistry. For visualization of whole skeletons, carcasses were dissected free of skin, viscera, and adipose tissue and fixed in 95% ethanol followed by acetone. Bone and cartilage were stained with 0.1% alizarin red S/0.3% alcian blue in 70% ethanol followed by clearing of tissues in potassium hydroxide in glycerol as described (18). For nondecalcified histology, bones were dissected free of soft tissues, sectioned at 5 μm, and stained with toluidine blue as described (19). For immunohistochemistry, dissected long bones and vertebrae were fixed in 2% paraformaldehyde/0.5 M lysine/0.01 M sodium periodate for 4 h at 4°C, washed in phosphate-buffered saline (PBS) for 1 h, and then infiltrated overnight with 30% sucrose in PBS. Tissues were subsequently quick-frozen, embedded in TBS tissue freezing medium (Electron Microscopy Sciences, Fort Washington, PA), and then sectioned by cryostat at 6 μm. Sections were then incubated as described (20) with rabbit anti-PTHrP (Oncogene Science) primary antibody at a 1:100 dilution followed by incubation with a fluorescein isothiocyanate-conjugated, anti-rabbit secondary antibody (Boehringer Mannheim). Sections were examined with a Zeiss Axiovert 10 confocal laser scanning microscope and a Bio-Rad MRC 600 imaging system using an optical slice thickness of 1–2 μm. Images consisting of nine scans per optical slice were collected on an optical memory disc and computer enhanced with Adobe PHOTOSHOP software for the determination of maximum pixel intensity per cell.

RESULTS

Overexpression of PTHrP in Chondrocytes of Transgenic Mice. The PTH/PTHrP receptor is expressed on immature

chondrocytes early in endochondral bone formation and on prehypertrophic chondrocytes in the growth plate (5). The mouse type II collagen promoter was, therefore, used to target expression of PTHrP to proliferating and prehypertrophic chondrocytes, with the assumption that it would be capable of delivering PTHrP to these receptor pools throughout endochondral bone development. Using standard techniques, three transgenic founder mice were generated. One founder failed to reproduce, whereas the other two gave rise to individual true-breeding lines that manifested qualitatively similar phenotypes.

The inclusion of human growth hormone sequences in the col II–PTHrP construct provided a convenient tag for assaying the level of transgene expression. RNase protection analysis of total RNA prepared from the forelimbs, hindlimbs, and tails of transgenic mice and nontransgenic littermates indicated that expression of the transgene was restricted to transgenic animals (Fig. 1*B*) and was approximately equal in the three sites examined. Additionally, there was no significant difference in level of expression of endogenous PTHrP or of the PTH/PTHrP receptor between transgenic and normal mice (Fig. 1*B*).

The location and degree of PTHrP overexpression in transgenic mice were examined by confocal immunohistochemistry performed on long bones of 5-day-old mice. As shown in Fig. 1*C*, while PTHrP was detectable in proliferating and prehypertrophic chondrocytes of normal mice, the intensity of labeling was clearly higher in the same cells of the transgenics, thus confirming that PTHrP is overexpressed in the expected location. To quantitate the magnitude of overexpression, mean pixel intensity of staining was examined within a defined chondrocyte population of transgenic and nontransgenic mice and indicated an approximately 4-fold increase in PTHrP expression in transgenic animals [pixel intensity for 35 cells (mean ± SD): normal mice, 14.7 ± 7.4; transgenic mice, 56.8 ± 10.3; $P < 0.001$].

Gross and Subgross Phenotype of col II–PTHrP Transgenic Mice. At birth, transgenic mice were readily distinguishable from nontransgenic littermates. Characteristically, they were small in size and manifested marked disproportionate foreshortening of the limbs and tail (Fig. 2). While transgenic mice appeared to grow at a rate comparable to their normal littermates, these phenotypic abnormalities were maintained throughout the life of the animals.

To examine the gross appearance of the skeletons, neonatal and 3-week-old mice were stained with alizarin red and alcian blue for bone and cartilage, respectively, to allow visualization of the skeleton. As shown in Fig. 3 *A* and *B*, there was a striking paucity of ossification throughout the skeleton of neonatal transgenic mice. Consistent with the col II-driven expression of PTHrP in chondrocytes, all skeletal elements that normally form by endochondral ossification were affected. Thus, in the skull, small centers of ossification were evident only in the basioccipital and exoccipital bones, whereas the remaining bones of the chondrocranium persisted as cartilaginous primordia. By contrast, those cranifacial bones that develop by intramembranous ossification appeared normal.

In the vertebral column, ossification centers were present in the dorsal arches of the cervical and thoracic vertebrae but were absent from the dorsal arches caudal to this point and from the vertebral bodies throughout the vertebral column. By contrast, in normal littermates, ossification of the dorsal arches and vertebral bodies was evident throughout the vertebral column and as far caudally as the midtail.

FIG. 2. Gross phenotype of 25-day-old transgenic and nontransgenic mice. The lower mouse is transgenic and the upper mouse is a normal littermate.

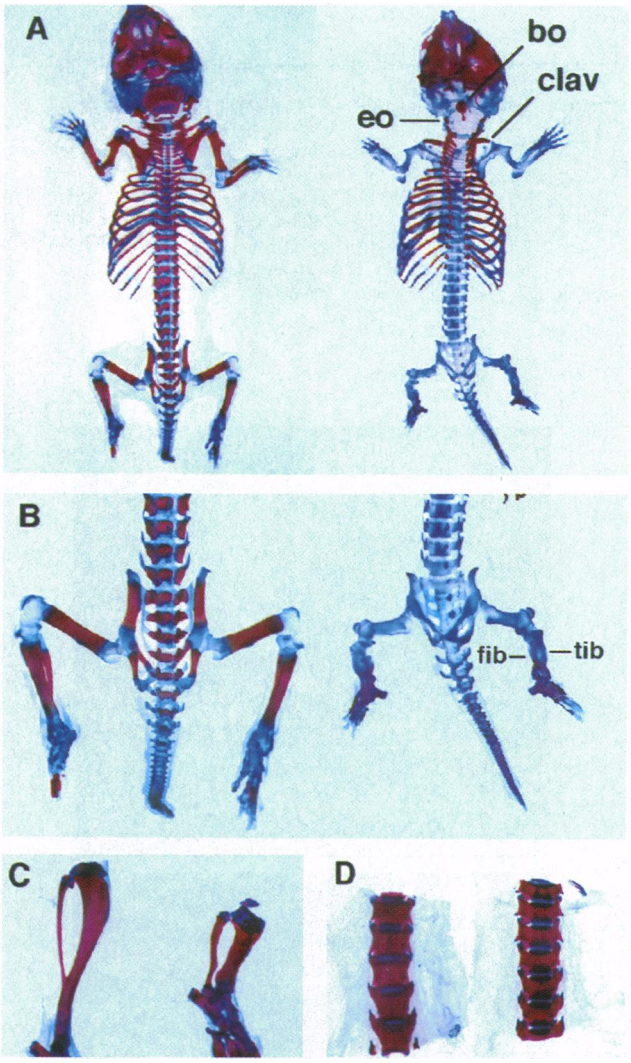

FIG. 3. Subgross analysis of skeletons of transgenic mice. Neonatal (*A* and *B*) and 3-week-old (*C* and *D*) mice were stained with alizarin red/alcian blue for bone and cartilage, respectively, followed by alkaline digestion to allow visualization of the skeleton. Transgenic mice are shown on the right and normal littermates on the left. (*A*) Ventrodorsal view of intact skeletons, showing a profound absence of ossification in the transgenic mouse. Early centers of ossification are evident in the basioccipital (bo) and exoccipital (eo) bones of the chondrocranium and in the ribs. Ossification is more advanced in the clavicles (clav). The degree of dwarfing of the transgenic mouse is not appreciated as it was photographed at a higher magnification than the nontransgenic. (*B*) Details of pelvis, hind ,limbs and lumbosacral and tail vertebrae in neonatal mice. Shortening and bowing of long bones is particularly evident in the tibia (tib) and fibula (fib). Metaphyseal flaring is also evident. (*C* and *D*) Long bones and lumbar vertebrae, respectively, of 3-week-old mice showing persistent shortening and deformities of the limbs and decreased vertebral length.

Other components of the axial skeleton, including the scapulae, pelvic girdle, and sternebrae, showed no evidence of ossification, and ossification centers, while present at the angles of the ribs, were clearly less developed in transgenic mice than in their normal littermates. Ossification in the clavicles, on the other hand, appeared to be quite advanced in the transgenic mice. This may reflect the very early appearance of centers of ossification in the clavicle (21) as well as the clavicle's complex pattern of ossification, which is both intramembranous and endochondral (22).

In the limbs of neonates, consistent with our findings in the axial skeleton, there was complete absence of ossification throughout. In addition, there was extreme shortening and bowing of the long bones, most evident in the tibia and fibula, and marked widening at their ends (Fig. 3*B*). These skeletal deformities persisted in the 3-week-old animals and accounted for the short-limbed phenotype observed (Fig. 3*C*). Further, at 3 weeks, vertebral length was clearly reduced throughout the vertebral column, accounting for the short tails and, in part, for the small stature observed in the transgenic animals (Fig. 3*D*).

Thus, our findings in the axial and appendicular skeleton indicated that endochondral ossification was apparent only at those sites that normally ossify early in gestation. This suggested that the normal sequence of ossification was maintained in the transgenic mice and, therefore, that overexpression of PTHrP was associated with a delay rather than a frank failure of ossification. In support of this suggestion, alizarin red/alcian blue staining of 3-week-old mice indicated a near-normal pattern of ossification throughout the skeleton (Fig.3 *C* and *D*).

Histologic Analysis. Characteristic histological features of normal neonatal long bones are shown in Fig. 4*A* and include a cortex, a well-developed marrow cavity with primary spongiosa adjacent to the growth plate, and cartilaginous epiphyses. By contrast, consistent with the appearance of the alizarin red/alcian blue-stained skeletons, in neonatal transgenic mice, the long bones showed no indication of bone formation but consisted of proliferating and prehypertrophic chondrocytes embedded in a cartilaginous matrix (Fig. 4 *B* and *C*). By 1 week, while no osseous tissue was yet evident, chondrocyte differentiation was apparent. During the normal program of differentiation, hypertrophy begins in the midsection of the cartilaginous primordia central to the developing periosteal bone collar and extends toward the extremities of the bone. By contrast, in the transgenic mice, hypertrophy appeared to initiate at the circumference of the long bone, both laterally and at the extremities, and formation of the bone collar was lacking (Fig. 4*D*). This suggested not only a marked delay in chondrocyte differentiation but also a disarray in the normal progression of events.

This pattern of ossification was clearly evident by 2 weeks of age, as shown in Fig. 4*E*. Cartilage in the periphery was replaced by primary spongiosa containing scattered remnants of cartilage and persisting in the center was a core of cartilage, with evidence of chondrocyte differentiation at the periphery. At 3 weeks, the long bones had largely been replaced by osseous tissue, although irregular areas of cartilage persisted, forming what might be called a pseudogrowth plate (Fig. 4*F*). Bone was woven, and in contrast to nontransgenic littermates (Fig. 4*G*), there was no evidence of cortex, marrow cavity, or parallel trabeculae adjacent to the growth plate. At higher magnification, osseous tissue adjacent to areas of cartilage appeared to be very active, with abundant osteoblasts, osteoid, and osteoclasts as well as evidence of fibrosis in marrow spaces (Fig. 4*H*). Further from cartilaginous cores these changes became less striking, and bone in the epiphyses was seemingly normal.

By 7 weeks of age, the long bones of transgenic mice appeared to have essentially normalized histologically, with the exception of some irregularity and thickening in the growth plates, which probably represented remnants of the cartilaginous cores seen at 3 weeks (Fig. 4*I*). Cortical bone, a marrow cavity, and parallel trabeculae adjacent to the growth plate were now evident and were largely undistinguishable from those seen in nontransgenic littermates (data not shown).

DISCUSSION

On the basis of previous findings and those described in the text, PTHrP appears to regulate the rate of programmed chondrocyte differentiation in developing endochondral bone as well as at the level of the growth plate (8, 23–26). Targeted disruption of the PTHrP gene results in a form of chondrodysplasia that leads to death at or shortly after birth (8). Grossly, these mice have a misshapen skull and short-limbed dwarfism, and histologically, they display a disordered growth plate characterized by a reduction in the columns of proliferative chondrocytes and premature/promiscuous ossification throughout the skeleton (8, 26). In the developing PTHrP-null fetus, skeletal abnormalities are seen in long bones as early as 14.5 days after conception, and the entire dysplastic process has been interpreted as resulting from an accelerated program of chondrocyte differentiation (8, 25, 26). This interpretation is supported by a number of experiments *in vitro* in which the addition of PTHrP or PTH to cartilage culture systems has been found to retard chondrocyte differentiation, as indicated by suppressed matrix calcification and type X collagen expression, and to inhibit apoptosis (refs. 24 and 25 and references therein). The older literature attributed such effects to PTH (ref. 24 and references therein), a supposition that is unlikely for many reasons, including the poor vascular supply of cartilage. Another intriguing recent finding bearing on putative PTHrP effects on chondrocytes is the report of an activating mutation in the PTH/PTHrP receptor in an individual with Jansen-type metaphyseal chondrodysplasia (JMC) (27, 28). The abnormalities in patients with JMC include hypercalcemia and hypophosphatemia associated with activation of classical PTH/PTHrP receptors on osteoblasts and in the kidney, respectively. Dysplastic findings associated with these receptors on chondrocytes include short-limbed dwarfism, dumbbell-like deformities of the foreshortened long bones, and histologically, retention of masses of cartilage in the metaphyseal regions in place of a growth plate (28). The metaphyseal abnormalities appear to heal by the late second decade, leaving a misshapen adult skeleton and the aforementioned biochemical abnormalities (27, 28).

The col II–PTHrP mice display an apparently unique form of chondrodysplasia that has two principal features. The first is a delay in chondrocyte differentiation and endochondral ossification so profound that the mice are born with a cartilaginous endochondral skeleton, clearly reflecting PTHrP's effects early in endochondral development. The second feature is short-limbed dwarfism with bowing and dumbbell-like deformities of long bones and a profoundly altered sequence of endochondral bone development. The histological findings in the col II–PTHrP mice at 7, 14, and 21 days of age revealed chondrocyte differentiation and the subsequent formation of primary spongiosa in a complete circumference around the central core of undifferentiated chondrocytes. This may reflect, in part, a gradient in the concentration of PTHrP that falls off as a function of distance from the cartilagenous core and, in part, a decrease in collagen II expression and hence PTHrP synthesis as chondrocytes progress toward hypertrophy. This unusual developmental sequence, in which a circumferential ossification center appears to replace the normal pattern of primary ossification, seems to lead initially to the formation of pseudogrowth plates that function poorly in terms of linear growth compared with true growth plates. By 7 weeks, a structure more closely resembling a normal growth plate forms, suggesting the existence of some patterning signal that specifies the formation of this structure at the epiphyseal–

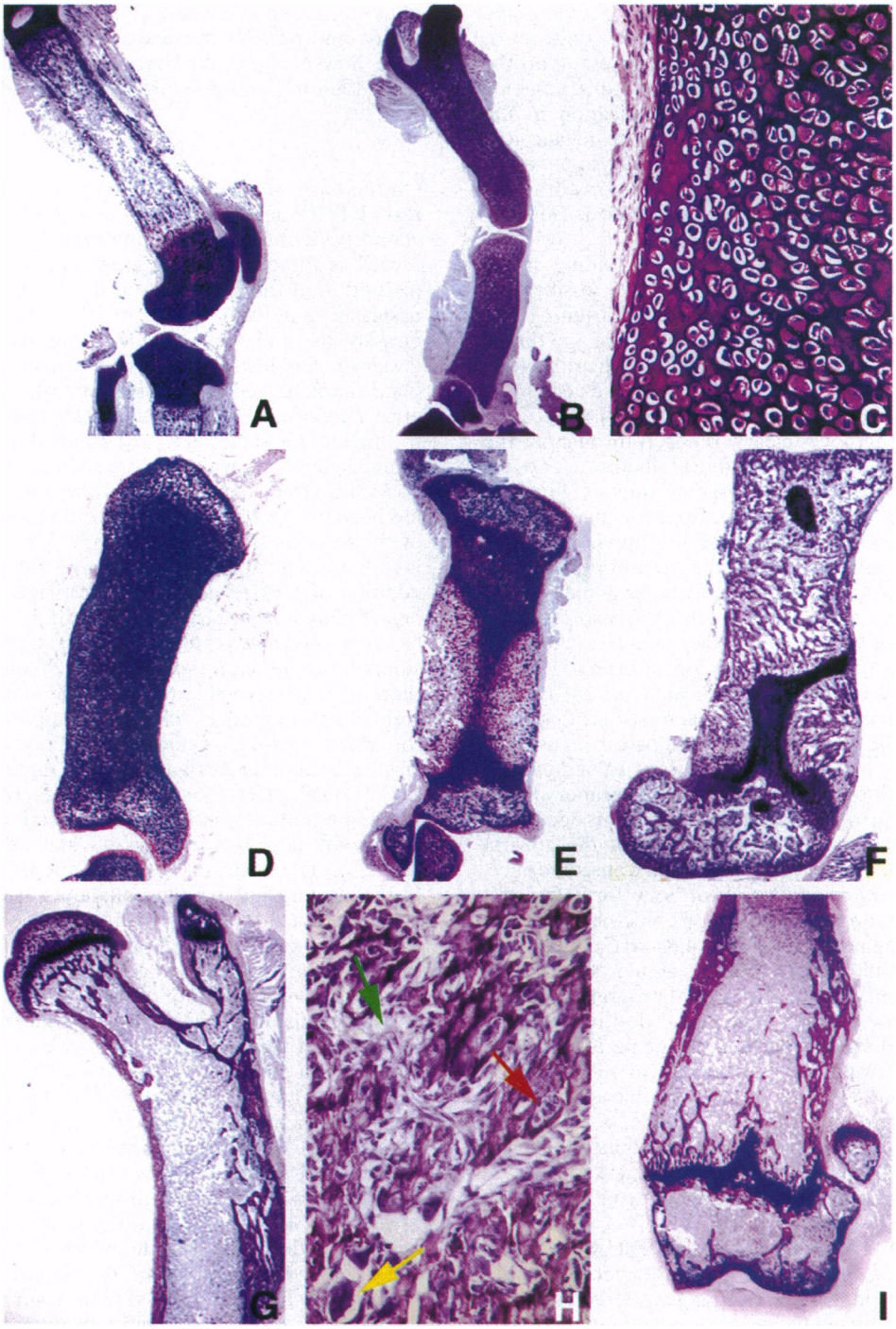

FIG. 4. Histological analysis of long bones of transgenic (*B–F, H,* and *I*) and normal (*A* and *G*) mice. All sections were nondecalcified and stained with toluidine blue. (*A–C*) Photomicrographs of the femur of normal (*A*) and transgenic (*B* and *C*) neonatal mice at low (*A* and *B*) and high magnification (*C*). (*D*) Tibia of transgenic mouse at 1 week of age. Contrary to the normal pattern of endochondral ossification, chondrocyte differentiation is beginning around the periphery of the long bone, as indicated by the presence of prehypertrophic and hypertrophic chondrocytes. (*E*) Section of femur at 2 weeks old. Primary spongiosa at the periphery and a persistent cartilaginous core are evident, further indicating a reversal in the normal program of ossification. (*F*) Femur of transgenic mouse at 3 weeks. Skeletal tissue has largely been replaced by woven bone. Irregular areas of cartilage persist, forming a highly irregular pseudogrowth plate. Cortex and marrow cavity are absent. (*G*) Femur of nontransgenic mouse at 3 weeks. Note the cortex, marrow cavity, and growth plate. (*H*) High-power photomicrograph of primary spongiosa in the femur of a 3-week-old trangenic mouse. Osteoblasts (red arrows), osteoclasts (yellow arrows), and osteoid are abundant, as are fibroblasts and fibrous tissue (green arrows). While these changes resemble osteitis fibrosa, the mice were normocalcemic and circulating PTHrP was undetectable. (*I*) Femur of a transgenic mouse at 7 weeks of age. The long bone has essentially normalized, except for some residual irregularity and thickening in the growth plate. No histological changes occurred in any other tissues examined (heart, lung, spleen, liver, and kidney). (*A, B, D–G,* and *I*, ×14; *C*, ×45; *H*, ×224.)

metaphyseal border even in a developmental sequence as unusual as that seen in the col II–PTHrP mice.

The col II–PTHrP model may well be relevant to the pattern of abnormalities seen in Jansen-type chondrodysplasia. The similarities between the two include the foreshortened and the dumbbell-shaped long bones, the residual "pseudo" growth plate-like masses of cartilage in the metaphyseal region, and the ultimate histological healing. It is therefore tempting to speculate that long-bone formation in individuals with JMC might proceed in a sequence that is similar to that in the col

II–PTHrP mice. The similarities do not include hypercalcemia, which was neither anticipated nor seen in the col II–PTHrP mice. Hypercellularity and fibrosis reminiscent of osteitis fibrosa were noted intermixed with the primary spongiosa at weeks 2 and 3 (see Fig. 4) and presumably reflect high concentrations of PTHrP in the vicinity of the cartilaginous cores interacting with receptors on the osteoblasts and/or marrow stromal cells in the primary spongiosa. The primary spongiosa seemed dense and almost osteopetrotic in the mice at this point in time, but both the fibrosis and the excessive primary spongiosa cleared with time.

The findings in the col II–PTHrP mice also provide insight into the apparent physiological regulation of chondrocyte differentiation by PTHrP, particularly when coupled with the findings in the PTHrP-null mice and in individuals with JMC, and with the temporal and spatial expression of the PTHrP and the PTH/PTHrP receptor genes (5, 8, 24, 27, 28). PTHrP would appear to act as a brake on chondrocyte differentiation via the type of gradient that typifies many developmental regulatory molecules, and as a result, cells closest to the PTHrP source would be developmentally retarded. If this interpretation is correct, early in normal bone development the PTHrP produced in the perichondrium and early chondrocytes of the cartilaginous mold, particularly at the ends, would be capable of regulating the rate of differentiation of chondrocytes in close proximity, leading to the normal ossification sequence that begins at the midshaft.

The apparent regulatory role of PTHrP in chondrocyte differentiation is consonant in general terms with other developmental effects that have been attributed to the peptide. Overexpression of PTHrP in the basal epithelial elements in skin results in a delay/failure of hair follicle development (6), and overexpression of PTHrP in myoepithelial cells in the breast is associated with a delay/failure in branching morphogenesis of the mammary epithelium (7). Thus, in all examples described to date, PTHrP appears to act relatively late in developmental time (e.g., the skin and breast phenotypes are postnatal), in all cases PTHrP seems to retard the progress of developmental programs, and in all cases paracrine pathways seem to be involved.

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