Clinic Rev Bone Miner Metab (2014) 12:104–118
DOI 10.1007/s12018-014-9163-8

ORIGINAL PAPER

Historical Perspective and Evolutionary Origins of Parathyroid Hormone-Related Protein

Janine A. Danks · Adam N. Freeman · T. John Martin

Published online: 22 March 2014
© Springer Science+Business Media New York 2014

Abstract After the discovery of parathyroid hormone-related protein (PTHrP) as the cause of the hypercalcemia of malignancy, it was found to be distributed widely in tissues, with its actions driving many physiologic and pathologic conditions. Its involvement in cancer extended to a contribution to the ability of cancer cells to promote bone resorption and establish as metastases. It was found to have multiple activities within the sequence, including a nuclear localizing sequence and a specific nuclear transport system. PTHrP and parathyroid hormone (PTH) appear to have arisen from a common ancestral gene and the comparative endocrinology and genomics studies focused on finding where the two genes appeared. PTHrP has been identified in bony and cartilaginous fish in the same tissues as in humans, indicating that PTHrP has fundamental and basic physiological roles in all vertebrates. PTHrP has been localized in fish neoplasms suggesting that PTHrP's role in tumor formation is a conserved role from at least fish to humans. Interestingly, PTH has been identified in both bony and cartilaginous fish even though they lack a parathyroid gland and indicate that PTH's evolutionary history is much longer too. So the point where PTHrP and PTH were duplicated is still unknown. A comparison among the analogous human, mouse, chicken, xenopus, and fugu sequences of the PTHrP gene demonstrates elements of conservation. When coupled with human Encode data and knowledge of interspecies gene structure, it offers rudimentary insights into function and underwrites hypotheses on physiology.

J. A. Danks (✉)
School of Medical Sciences, RMIT University,
PO Box 71, Bundoora, VIC 3083, Australia
e-mail: janine.danks@rmit.edu.au

J. A. Danks
Department of Medicine, Austin Health, University of Melbourne, Heidelberg, VIC 3081, Australia

A. N. Freeman
Department of Surgery, St Vincent's Hospital, University of Melbourne, Fitzroy, VIC 3051, Australia

A. N. Freeman
Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, University of Melbourne, Parkville, VIC 3052, Australia

T. J. Martin
Department of Medicine, St Vincent's Hospital, University of Melbourne, Fitzroy, VIC 3051, Australia

T. J. Martin
St Vincent's Institute of Medical Research, Fitzroy, VIC 3051, Australia

Keywords Parathyroid hormone-related protein · Parathyroid hormone · Cancer · Fish · Shark · Elephant shark

Abbreviations

PTHrP	Parathyroid hormone-related protein
PTH	Parathyroid hormone
HHM	Humoral hypercalcemia of malignancy
PTHLH	*Parathyroid hormone like hormone* (the gene encodes the PTHrP protein in humans)
Pthlh	*Parathyroid hormone like hormone* (the gene encodes the PTHrP protein in rodents)
PTH1R	Parathyroid hormone 1 receptor
pthrp	*Parathyroid hormone-related protein* (the gene that encodes the Pthrp protein in fish and amphibia)
VMA	Vertebrate Multiz Alignment
UTR	Untranslated region
CcN	CK2-cdc2-NLS motif

NLS Nuclear localization sequence
NFT2 Nuclear transport factor 2

Introduction

Parathyroid hormone-related protein (PTHrP) was discovered in the search to identify mechanisms by which certain solid cancers cause hypercalcemia without metastasizing to bone [1, 2]. This syndrome had for a long time been ascribed to inappropriate production by tumors of parathyroid hormone (PTH), a concept that arose from a suggestion by Albright [3] in discussing a patient with renal cancer and elevated calcium and lowered phosphorus. Since this patient had no apparent parathyroid cancer, Albright correctly theorized that the high plasma calcium and low phosphorus were the result of tumor elaboration of a PTH-like substance. This novel idea appeared to be supported two decades later with the development of the first PTH radioimmunoassay that identified elevated plasma PTH in patients with bronchogenic carcinoma [4]. The concept of "ectopic PTH production" by cancers was accepted through the 1960s (e.g., [5]), but the idea began to lose favor by the 1970s.

Some doubt about this view began to emerge when more specific radioimmunoassays were developed. Two groups independently identified that the circulating PTH in hypercalcemic cancer patients was immunologically distinguishable from human PTH [6–8] and that the plasma levels were lower than in patients with primary hyperparathyroidism. In addition, breast tumor extracts from a hypercalcemic patient contained activity that reacted with a specific PTH antiserum, but which was non-parallel to normal PTH standards [9], suggesting a related, but distinct antigen. When several different anti-PTH sera were used that recognized various epitopes, PTH could not be identified in a series of plasma and tumor samples from patients with hypercalcemic cancers [10]. Thus, it was concluded that the cancer-associated hypercalcemia was caused not by PTH, but by a different and distinct entity with many PTH-like effects, giving rise to the term "humoral hypercalcemia of malignancy" (HHM) [11]. Affirmation of this came from a number of excellent clinical studies showing that patients with hypercalcemia in cancers that had not metastasized had low plasma phosphate levels, increased nephrogenous cyclic AMP and suppressed PTH [12–14].

It had become clear that certain crucial biochemical actions were shared by PTH and the unknown related substance. These included promotion of bone resorption sufficient to cause hypercalcemia, as well as promotion of the renal excretion of phosphorus and of the generation of nephrogenous cyclic AMP. Based on the latter response, rapid, sensitive, robust biological assays of PTH activity had been developed [15–17]. These were applied to the identification of PTH-like activity in tumor extracts from HHM patients [18], in tissue culture medium [19] and in animal models of HHM [20]. A sensitive cytochemical assay was also used to show elevated levels of PTH-like activity in the plasma of HHM patients that was distinct from PTH [21].

Identification of PTHrP and Its Gene

Driven by the clinical studies described above, and the availability of biological assays with high sensitivity and specificity, and capable of high throughput, the efforts to isolate and clone of the PTH-like activity followed. Certain criteria were applied to identification of PTH-like activity in tumor extracts and media. These were (1) it should promote adenylyl cyclase activity strictly in PTH targets, (2) that action should be inhibited by peptide receptor antagonists of PTH–receptor interaction, but not by anti-PTH neutralizing antiserum, and (3) PTH should be undetectable by radioimmunoassay in extracts or media. These stringent requirements were met in several early studies of PTH-related activity identified in tumor extracts from patients and animals with HHM [18, 20, 21] and in culture media from one such tumor line [19].

Using a cell line from a patient with HHM in association with a squamous cell carcinoma of the lung, PTHrP was purified and sequenced through the first 16 amino acid residues, revealing that 8 of the first 13 residues were identical with those in PTH, but no PTH was expressed by the cells. Anti-peptide antibodies based on this short sequence were able to detect PTHrP but did not cross-react with synthetic PTH(1–34) [1]. Further purification allowed sequencing of the first 50 residues to be obtained [2]. Purification was also achieved from a breast cancer extract [22] and a renal cancer cell line [23]. Cloning revealed a pre-pro-peptide of 36 amino acids and a mature protein of 141 amino acids with significant homology with PTH about the N-terminal region [2, 24], but no more identities within the remainder of the sequence other than those expected by chance. The biological activity of PTHrP at the PTH receptor was contained within the first 34 residues, and study of the efficacies of several truncated peptides showed that the requirements for biological activity within the amino-terminal region of this new protein were very similar to those within PTH [25].

Data rapidly accumulated to show that PTHrP was expressed by squamous cell cancers regardless of origin, as

well as in a number of other tumors, including renal cortical carcinoma, breast cancer and neuroendocrine tumors. The part played by PTHrP in these cancers and in bone metastases will be discussed by Guise and co-authors in this volume. PTHrP was identified by immunohistochemistry in several non-malignant tissues, including keratinocytes, endothelial cells, smooth muscle, bone, lactating mammary tissue and neuroendocrine tissues [26], as well as in mammalian embryonic tissues [27].

PTHrP as a Multifunctional Cytokine

Although PTHrP was discovered as a hormone produced in excess by certain solid tumors, its physiological functions result predominantly from its action as a paracrine, and perhaps as an autocrine or even intracrine manner in multiple tissues. Only in three circumstances has PTHrP been shown convincingly to be measurable present in the circulation and acting in an endocrine fashion. These are (1) the HHM syndrome, in which PTHrP is secreted by tumors and acting systemically, (2) lactation, in which PTHrP is made in the breast and reaches the circulation, and (3) fetal life, where PTHrP regulates maternal-to-fetal placental calcium transport. Since *PTHLH* mRNA is highly unstable and its expression regulated by multiple growth factors and cytokines with which it is often co-expressed (during development and in various tissues), it rapidly became regarded as a cytokine.

Other chapters will engage in detail with the local roles of PTHrP in many tissues, including bone and cartilage, vascular smooth muscle, placenta, breast development and pancreatic islets. Introductory comments are provided here to consider how PTHrP might act locally in mammalian tissues, before discussing its identification and possible roles in non-mammalian species.

With PTH as a circulating hormone, and since the parathyroid glands only develop after mesenchymal condensations are formed at sites of skeletal development, it is the paracrine/autocrine factor PTHrP, secreted locally by chondrocytic cells, that plays the prime role in endochondral bone formation. Deletion of *Pthlh* in mice resulted in perinatal death from respiratory failure due to defective rib development [28, 29]. Rescue of these mice by directing PTHrP production to cartilage using the collagen II promoter allowed study of the PTHrP-null phenotype in several other organs. Thus, the central role of PTHrP in endochondral bone formation was discovered [30, 31], with PTHrP exerting a paracrine role, produced by the distal perichondrium and interacting with PTH/PTHrP receptor (PTH1R) in the proliferative and hypertrophic zones of the growth plate, revealing it to have a major developmental role.

The predominant role of PTHrP in bone metabolism was demonstrated in mice deficient in PTHrP. Despite the neonatal lethality of *Pthlh* (−/−) mice [29], haploinsufficient *Pthlh* (±) mice, that are phenotypically normal at birth, by 3 months of age have low bone mass, with a marked decrease in trabecular thickness and connectivity, and an abnormally high number of adipocytes in the bone marrow compared to wild-type littermates [32]. The critical role of osteoblast-derived PTHrP in the process of bone formation was confirmed when this phenotype was recapitulated in transgenic mice with osteoblast-specific deletion of *Pthlh* [33]. Importantly, mice with *Pthlh*-deficient osteoblasts were normocalcemic, emphasizing the fact that PTHrP action in bone is not required for the maintenance of calcium homeostasis.

One of the most clearly defined physiological actions of PTHrP has been its role as a local paracrine regulator of smooth muscle tone. It had long been recognized that injection of PTH (or parathyroid extract in the early years) rapidly increased blood flow through a range of vascular beds, accompanied by decreased blood pressure [34–36] (reviewed in [37]). PTHrP was found to be produced in vascular smooth muscle cells and to be rapidly up-regulated in response to mechanical distension of the arterial wall, to increases in arterial pressure, and to vasoconstrictors such as angiotensin II [38, 39]. It interacts with the PTH1R, acting as a potent relaxing agent for cardiac and smooth muscle from many locations such as blood vessels, uterus and the gastrointestinal tract [37, 40, 41]. Thus, the pharmacological effects previously observed with PTH resulted from widespread activation by PTHrP of PTH1R receptors in vascular beds [42, 43].

Although there is ample evidence for the cardiac, blood vessel and other smooth muscle actions of PTHrP, the *Pthlh*-null mutant mice develop normal cardiovascular systems [28], indicating that PTHrP is not essential for cardiovascular development. However, because the animals die at birth of skeletal and perhaps other defects, the roles of PTHrP in vascular remodeling, local regulation of vascular flow and the vascular response to injury in adult animals remain undefined.

Nuclear Import and Intracrine Function of PTHrP

PTHrP appears so far to be the only protein classed at least in some circumstances as a hormone, which possesses a candidate nucleus (CcN) motif and displays differential cellular localization (nuclear/nucleolar versus cytoplasm). The functions served by nuclear/nucleolar localization of PTHrP remain obscure, but it is such a striking property that it needs to be borne in mind in considering what roles PTHrP might play as an autocrine or intracrine mediator.

This might become all the more important in investigating in due course how PTHrP functions physiologically in fish and in even more primitive species, where it seems likely that it will exert its action more as a local than as a humoral mediator.

Nuclear location of immunoreactive PTHrP was observed in 5–10 % of transiently transfected smooth muscle cells [44], and in chondrocytes, its nuclear localization was associated with enhanced chondrocyte survival following prolonged periods of serum starvation [45]. The localization was associated with a highly basic region within PTHrP (67–93). Studies in both smooth muscle cells [46] and keratinocytes [47] have demonstrated PTHrP expression to be cell-cycle-dependent. The highest levels of PTHrP mRNA appeared to be a response to mitogenic factors only at the G1 phase of the cell cycle during which PTHrP localized to the nucleolus. PTHrP also localizes to nucleoli where its role is still not understood [48]. This is likely mediated by a nucleolar localizing sequence from (94–108), immediately after the NLS [44]. Nucleolar localization has been shown to be cell type and cell cycle dependent, and may be a component of cell cycle control. For instance, it is increased in response to smooth muscle cell proliferation [49].

Within the PTHrP sequence, there are CK2-cdc2-NLS (CcN) and nucleolus localization motifs, with the former being similar to that described for the archetypal CcN-containing protein, SV40 T-antigen [50], comprising also consensus protein kinase CK2 and confirmed cyclin-dependent kinase phosphorylation sites. This similarity in structure led to experiments showing specific import of PTHrP into the nucleus, brought about by binding to importin β, which together with the GTP-binding protein Ran is able to mediate efficient nuclear accumulation in the absence of importin α, whereas addition of nuclear transport factor 2 (NTF2) reduced transport [51]. In support of these observations, the crystal structure showing importin β bound to the nuclear localizing sequence of PTHrP has been determined [52]. This novel nuclear import mechanism for PTHrP may be the result of either an intracrine pathway in a cell expressing PTHrP, or in a target cell as a result of binding of PTHrP to the PTH1R and internalization of the complex, leading to nuclear import; there is evidence supporting each of these potential pathways.

First, the idea that intracrine action is a feasible prospect comes from studies showing altered translation initiated from sites downstream of the initiator methionine of PTHrP [48]. This would give rise to forms that bind weakly or not at all to endoplasmic reticulum, and thus be available for transport to the nucleus [48, 49, 53]. The ability of *PTHLH* to be translated from start sites other than AUG codons was the first to be described in eukaryotes.

Second, internalization of PTHrP (1–108) using a fluorescein isothiocyanate label could be observed only in cells expressing the PTH1R [54]. Moreover, transport of PTHrP(1–141) to the nucleus after binding to the cell surface required an intact NLS [44]. The latter effect was abrogated by pre-treatment with PTHrP(74–113) alone, indicating the involvement of another cell surface receptor in endocytosis-dependent nucleolar translocation. Such receptor-dependent nuclear translocation of PTHrP, similar to mechanisms operating with interleukin-5 and growth hormone [55], as well as vascular endothelial growth factor [56], implied that the PTH1R should be found in the nucleus. There is some evidence for that, with the PTH1R being found by immunostaining in the nucleus of cell lines and rat tissues [57, 58]. Furthermore, the cytoplasmic tail of the PTH1R at residues (471–487) has a putative NLS similar to that of PTHrP [58].

PTHrP Post-translational Processing: Active Domains

In addition to the complex transcriptional control of *PTHLH* gene expression, the individual isoforms themselves are subject to a variety of critical post-translational modifications and enzymatic processing. The first 36 amino acids (−36 to −1) code for the intracellular "prepro" and "pro" precursors of the mature peptide which are important for intracellular trafficking and secretion of polypeptide hormones [2]. The 14–36 region of PTHrP which has almost no homology with the primary amino acid sequence of PTH (Fig. 1), nevertheless appears to be critical for the binding of PTHrP to the PTH1R receptor and for its subsequent activation [25, 59].

The importance of the next region extending from amino acids 36–139 (Fig. 1) is less well established. This region is encoded by all three isoforms of human PTHrP mRNA, and the primary sequence is highly conserved across species through to amino acid 111, indicating that important functions are potentially contained within this part of the molecule, though few have been definitively and repeatedly demonstrated. The "mid-molecule" portion, between residues 35–84, is responsible for promoting calcium transport across the placenta, making calcium available for fetal skeletal development [60–62]. PTHrP(107–139) has been reported to inhibit osteoclast activity and bone resorption in vitro [63] and in vivo [64], and to promote osteoblast proliferation and function [65–67], perhaps by down-regulation of sclerostin [68]. Although no receptor or signal transduction mechanism has been identified, preliminary evidence has suggested an action of PTHrP(107–139) through a Ca^{2+}-coupled receptor of unknown identity in osteoblasts [69]. These experiments with the mid-region and distal regions of PTHrP are reminiscent of similar studies

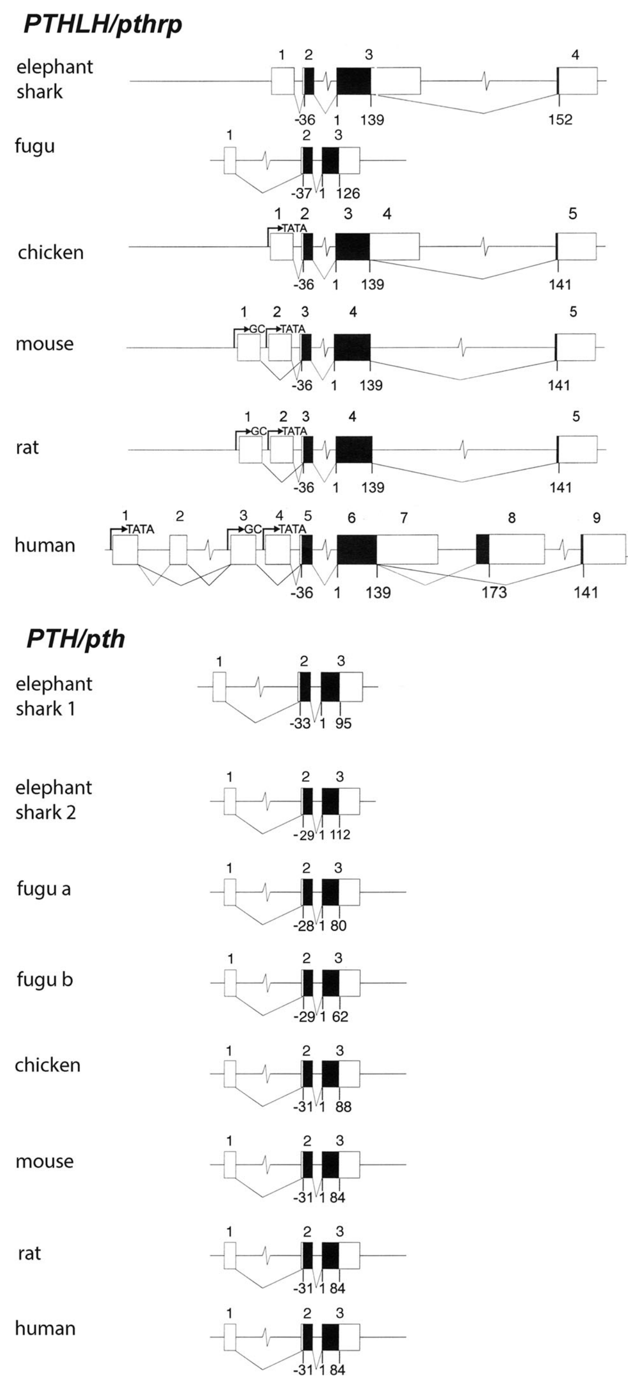

Fig. 1 A comparison of the gene structure of elephant shark, *Fugu*, chicken, mouse, rat and human *PTHLH/pthrp* and elephant shark, *Fugu*, chicken, mouse, rat and human *PTH/pth* (adapted from Liu et al. [104])

with carboxyl fragments of PTH, where biological effects have been identified [70], but no mechanism(s) ascertained.

Genetic experiments in mice support the likelihood that other biological activities may be exerted by domains within PTHrP that are released by proteolytic cleavage. The knock-in of PTHrP (1–84) lacking both the nuclear localization sequence (NLS) and C-terminal region while retaining the bioactive amino-terminal region, resulted in multiple abnormalities and early lethality in mice [71]. Homozygous mice exhibited skeletal growth retardation and osteopenia associated with reduced proliferation and increased apoptosis of osteoblasts, and early senescence. In a second model, the knock-in of PTHrP(1–66), which excludes a significant portion of the mid-region, resulted in a similar, but even more severe phenotype [72]. These mouse studies convincingly demonstrate that many of the actions of PTHrP are not mediated solely by the amino-terminal region, and that the absence of the mid-region, NLS or C-terminal region of PTHrP, result in greatly impaired commitment and survival of both osteogenic and hemopoietic precursors. Additional studies are required to ascertain the mechanisms of action of these important but less well-defined PTHrP regions. The final C-terminal "tail" region of PTHrP, from amino acids 142 to 173, is encoded by only one of the three isoforms of human PTHrP mRNA and is not represented at all in other species. The significance of this isoform remains unknown, although PTHrP(141–173) immunoreactivity has been identified in plasma [73], and PTHrP(140–173) immunoreactivity is present in human amnion [74]. Thus, while might be presumed that the actions ascribed to regions of the molecule beyond the N-terminal 34 amino acids are mediated by unique receptors, these have yet to be discovered.

Potential enzymatic processing sites abound in the PTHrP primary sequence, and it seems likely that processing of PTHrP takes place in an isoform- and tissue-specific manner. The physiological implications of post-translational processing of PTHrP are still poorly understood [75]. However, it is clear that the secreted forms of mid- and COOH-terminal regions of PTHrP have a number of bioactivities. This implicates PTHrP as a polyfunctional protein, as is the case with many neuroendocrine proteins that give rise to families of mature secretory peptides [76]. These forms may have their own receptors to elicit signaling pathways that are in part regulated by isoform- and cell-specific processing and secretion. It may also be that the great susceptibility of PTHrP to endoproteolytic cleavage is the result of evolutionary pressure to fit it so well as a paracrine and autocrine regulator with a very short tissue half-life and at the same time be a source of peptide products that serve other purposes, either as circulating hormones or as local agents.

The Mammalian PTHrP Gene and Its Products

The finding of multiple RNA transcripts on Northern analysis suggested that alternative splice products were likely. This was established with data showing two further

cDNA species, predicting PTHrP variants of 139 and 173 amino acids in length [77–79]. When the genomic structure was identified shortly after [79–81], all three promoter regions of the complex *PTHLH* gene were defined and their specific splicing patterns resolved and confirmed. In normal human cells, PTHrP is encoded by a single copy *PTHLH* gene located on chromosome 12p11.2–p12.1 [77, 81]. The *PTHLH* locus is distinct from that of the *PTH* gene, which is located on chromosome 11p15.4. Chromosomes 11 and 12 are thought to have arisen through duplication events from a single common ancestral chromosome [82]. In addition to *PTH* and *PTHLH,* there are several examples of related genes, one localized to chromosome 11 and a related gene present on chromosome 12 [82, 83].

The *PTHLH* and *PTH* genes are similar in their overall genomic organizations, having similar exon–intron boundaries (Fig. 1). The human *PTHLH* gene is composed of nine exons spanning approximately 15 kb. The gene is transcribed by three functionally distinct promoters, and primary mRNA transcripts are alternatively spliced both at the 5′ and 3′ ends (Fig. 1) [79–81, 84]. It should be noted that with this nomenclature, there is no intron between exons 6 and 7 in the human *PTHLH* gene, although the sequence of this junction represents a splice donor site consensus sequence, and the observed splicing involving this site and exons VIII or IX shows its operation as a splice donor site.

In their structural organizations, the chicken, mouse and rat *Pthlh* genes share substantial homology with the human *PTHLH* gene, although the mouse and rat genes are considerably simpler than the human gene. The greater complexity of the human *PTHLH* gene appears to be a late evolutionary event, especially in light of the relative simplicity of the mouse and rat genes (Fig. 1). Both have exons equivalent to exons 4, 5, 6 and 9 of the human *PTHLH* gene and do not undergo alternative 3′ splicing, thereby producing only one isoform of PTHrP (Fig. 1). This conservation suggests that these exons constitute a minimal *PTHLH* gene structure. There is no evidence to suggest that the mouse or rat have exons equivalent to exons 1 or 2 of the human *PTHLH* gene. To date only one promoter region has been defined for the mouse and rat genes, equivalent to the P3 promoter of the human gene. Nucleotide sequence comparison of the mouse and rat genes with the human gene demonstrated substantial homology over the GC-rich promoter region (P2) and exon 3 of the human gene, suggesting that this region is also transcribed in rodents. Confirmation of this idea was achieved when transcripts corresponding to human exon 3 were detected in a clonal rat line (PT-r) [85] and an HHM-associated rat Leydig cell tumor (H500) [86]. However, there is no evidence to suggest that the mouse or rat have exons equivalent to exons 1 or 2 of the human *PTHLH* gene.

Evolutionary Aspects of PTHrP: Identification in Fish

From the time of the discovery of PTHrP, an obvious question was posed—if PTH and PTHrP were derived from a common ancestral gene, evolving through gene duplication, which came first, and was one a copy of the other? The search for answers to these questions began in fish. At the time of the discovery of PTHrP, stanniocalcin had recently been sequenced in the eel [87]. Stanniocalcin is a hypocalcemic hormone which was first identified in fish [87] and subsequently isolated and cloned in mammals [88]. When antiserum against hPTHrP(1–14) was used on sections of eel corpuscles of Stannius, the corpuscles were negative but the adjacent kidney tubules stained positively. The corpuscles from a number of fish species were also examined immunohistochemically for PTHrP, and all were found to be negative [89]. PTHrP immunoreactivity was also found in the corpuscles of Stannius in the euryhaline flounder (*Platichthys flesus*) [90] and possibly related to the fish moving from fresh to saltwater as part of its lifecycle.

The positive PTHrP staining in kidney tubules prompted a more extensive examination resulting in the demonstration of PTHrP in a number of fish tissues. In addition to the kidney tubules, PTHrP was localized to two areas of the fish pituitary (*Sparus aurata*), primarily located in the pars intermedia, and there were high circulating levels in sea bream as measured by N-terminally directed radioimmunoassay [91]. The presence of the antigen in the pituitary was surprising, but that the late Parsons [92] had suggested at a conference in 1979 that cod pituitary contained a factor similar to, but distinct from PTH. Parsons had data that this factor was hypercalcemic and contained a region that was "immunologically closely similar to the amino-terminal" of mammalian PTH. He predicted that this region would be relatively invariant between species and also speculated that this fish hypercalcemic factor might be "primitive" and the "evolutionary history of PTH may be hundreds of millions longer than previously believed". This prescient statement was followed by the speculation that this hypercalcemic factor evolved as an anabolic agent because fish do not resorb their skeleton [92].

There were a number of studies that followed indicating that there was PTH or PTH-like activity in brain and pituitary of some bony fish [93, 94]. Other studies focused on tissues of fish and shark species and found that PTHrP localization reflected the picture seen in humans. Using antisera raised to amino-terminal hPTHrP, PTHrP antigen was demonstrated in the skin, kidney and muscle of lamprey (*Geotria australis*), lungfish (*Neoceratodus fosteri*),

Table 1 The parathyroid hormone family genes identified in a range of vertebrate and invertebrate species

Common name	Scientific name	*PTHLH/ Pthrp*	*PTH*	*PTH-L*	*PTH1R*	*PTH2R*	*PTH3R*
Human	*Homo sapiens*	+	+	–	+	+	–
Rat	*Rattus rattus*	+	+	–	+	+	–
Chicken	*Gallus gallus*	+	+	+	+	–	+
Amphibian	*Xenopus laevis*	+	+	+	+*	+	+
Teleost	*Fugu rubripes*	+*	+*	+	+	+	+
Elephant shark	*Callorhinchus milii*	+	+, + (*pth1* & *pth2*)	?	+	+	+
Lamprey	*Petromyzon marinus*	?	?	?	+	+	?
Sea squirt	*Ciona intestinalis*	?	?	?	+	+	?

+, one identified gene; ++, two separate genes; +*, duplicated genes; –, not present; ?, not known

seven species of elasmobranchs (sharks) and rainbow trout (*Oncorhynchus mykiss*) [95, 96]. This raised the possibility that PTHrP might have role(s) similar to those in mammals, probably acting in an autocrine/paracrine manner and influencing cell growth and differentiation. This was supported by the demonstration of both the gene and protein in the developing denticle in one of the shark species (*Mustelus antarcticus*) [95]. Elasmobranchs have a cartilaginous skeleton and include sharks, skates and rays. It has been asserted by a number of researchers that both modern bony and cartilaginous fish have a common bony (armored) ancestor and that remnants of this armor exist in the scales on bony fish and the dermal denticles found on the surface of sharks.

Using parallel immunostaining and in situ hybridization, expression of the *pthrp* gene was also localized in the same tissues of the lamprey, elasmobranchs and rainbow trout [95]. Both protein and mRNA were localized in liver of the lungfish, elasmobranchs and rainbow trout as with the rectal gland of the elasmobranch. Identification of Pthrp in the rectal gland implied that it might have a role in osmoregulation [95, 97, 98]. This study also showed that the Pthrp protein was present in the pituitary of the lungfish, elasmobranch and rainbow trout, and the gene was present in the elasmobranch and rainbow trout pituitaries. The protein was detected in the nerve chord of the bony and cartilaginous fish as well [95].

Cloning of Fish *PTHrP* Genes

Once whole genome sequencing of the bony fish *Fugu rubripes* began in early 1990s, efforts began to isolate and clone the fish *Pthrp* gene. These studies revealed that gene structure (Fig. 1) was simpler than that of chicken, mouse or human with only three exons [96]. This structure was more like the highly conserved three exon *PTH* gene and was an exciting finding because it suggested that we might be closer to the point in evolution that indicates the duplication event explaining the similarity between PTH and PTHrP. This had been predicted because these two genes are on short arms of chromosomes 12 and 11, respectively, and the gene order and synteny is highly conserved among vertebrate species. When the first fish *pth* gene was identified in *Fugu* [99], this was counter to the accepted dogma that amphibians were the first animals to have PTH as they are the most primitive species to possess a parathyroid gland. Indeed, it dispensed with the idea that a parathyroid gland was needed to produce PTH.

Subsequently, several other copies of fish *pthrp* and *pth* genes were located in the *Fugu* genome [100]. These authors identified two *pth* genes (*pthA* and *pthB*), two *pthrp* genes (*pthrpa* and *pthrpb*) and a Pth-like ligand (*pth-L*) (Table 1). They were found to have hypercalcemic activity in fish but these extra copies of both *pth* and *pthrp* genes are not present in the human genome [101]. Although *pth-L* has not been identified in mammals, it has been found in the genomes of frogs and chickens [101] (Table 1). These in silico experiments have suggested that gene structure of *Pth-L* is more like *pth* than *pthrp*.

It should be emphasized that bony fish have undergone a "teleost-specific" genome duplication. Genome duplication has long been accepted as the principal method by which new genes arise, resulting in new genes with different functions [102]. Some evidence implies that genome duplication allows the evolution of diversity [103]. The vertebrate genome has undergone several rounds of gene duplication, two before the jawless fish (agnathans), and then after bony fish split off from other vertebrates they underwent their "teleost-specific" genome duplication. This helps to account for the number of fish species worldwide and their ability to fill very different environmental niches. To further complicate matters, salmonids underwent another genome-wide duplication on their own, resulting in a large genome.

Both *pthrp* and *pth* genes were identified in the genome of the elephant shark (*Callorhinchus milii*) [104]. Two *pth* genes were present but only one *pthrp* gene (Fig. 1;

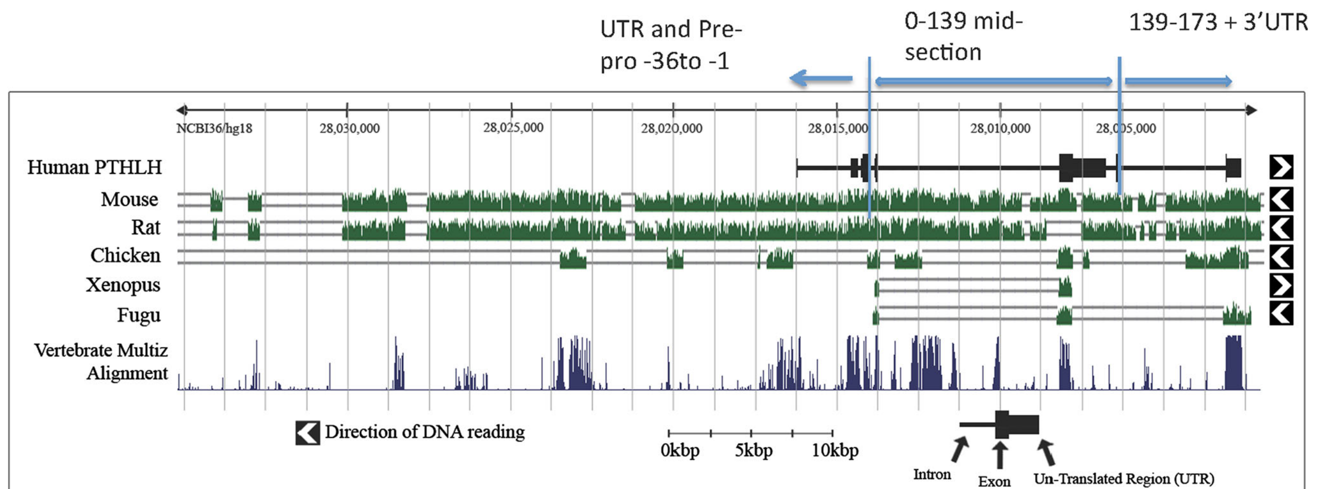

Fig. 2 Comparative genomics of the *PTHLH/pthrp* gene and its 20-kbp upstream region mapped to the HG18 genome (see text for details). The human *PTHLH* gene is featured in the *top row*, *mirrored horizontally* as it is an antisense gene. The *second to fourth rows* feature the genes for the mouse, chicken, *Xenopus* and *Fugu*. Where stretches of DNA comparative to the human gene exist, their conservation is graphed, leaving gaps where no comparable sequence exists. The Vertebrate Multiz Alignment graphs the relative conservation of each base pair among 28 vertebrate species. The *bottom row* features the results of longitudinal Encode H327Ac assay performed on multiple cell types

Table 1). Elephant sharks have a cartilaginous skeleton-like elasmobranchs but belong to a sister group, the chimeras. They are the oldest group of living jawed vertebrates, and their genome has been termed the "vertebrate reference genome" [105]. The elephant shark *pthrp* gene was expressed in a wide range of tissues including kidney, brain, eye, heart, muscle, ovary, testis and uterus, reflecting the patterns seen in mammalian tissues [104]. The elephant shark Pthrp protein was localized in a broad range of tissues mirroring not only the expression but also immunohistochemical findings in other studies in fish and humans. This conservation of localization through hundreds of millions of years indicates that the function has probably been conserved as well. Certainly elephant shark Pthrp(1–34) peptide activated the common PTH1R in the rat osteosarcoma cell line, UMR106 [104]. It had been shown previously that *Fugu* pthrp(1–34) could activate the Pth1r in fish scale assays [100]. Recent work by Yan et al. [106] showed that the knockdown of *zpthrpa* gene causes premature bone mineralization during craniofacial development in zebrafish.

Interspecies comparison among the analogous *PTHLH/Pthrp* sequences reveals interesting similarities and offers rudimentary insight into function. Figure 2 depicts *PTHLH* and approximately 20 kilobase pairs (kbp) 5′ in the human gene [107]. Homologous segments of the mouse, chicken, *Xenopus* and *Fugu* DNA are drawn for comparison [108]. A graph of the Vertebrate Multiz Alignment (VMA) plot, a score of base-wise conservation across 28 vertebrate species, affords a more global perspective on conservation [109] (Fig. 2). The Encode H3k27Ac signals from the region are also plotted as an indication of likely enhancer and promoter activity locations [110] (Fig. 2).

It should be noted that the segment of DNA is reversed in both *Xenopus* and human, *PTHLH* being an antisense gene, but not the others. This indicates a transposition or breakage must have occurred in speciation. At least the 20 kbp 5′ is generally considered regulatory of the adjacent gene. The mouse shares significant homology with the human in this region, while the chicken shares shorter segments and is similar to the VMA, a consensus sequence based on 28 vertebrate species. This relationship continues through the intronic regions of *PTHLH* proper, which are likewise regulatory.

Considering the gene in its functional divisions, zone 1 in Fig. 2 contains exons 1–3. It also includes the 5′ untranslated region (UTR) which is likely important for post-transcriptional trafficking and regulation. The bulk of this segment is shared in its entirety by the human and mouse, but not the others featured. The VMA shows that the adjacent UTR cap forming the start point of the gene is moderately conserved. The intronic segment immediately preceding exon 2, however, is particularly highly conserved throughout the VMA species, along with the two segments of UTR, which are only slightly less conserved. This intron is likely a promoter region, which is supported by the high Encode H3k27ac assay signal intensity.

The second zone includes several introns as well as exons 4–5. The first intron precedes exon 5 and is well conserved in the mouse and chicken, but not *Fugu*, *Xenopus* or the VMA group. It likewise shows signs of regulatory potential on the H3k27Ac assay. Exon 5 contains

further a UTR as well as the pre-pro sequence (−36 to −1) for entry to the endoplasmic reticulum and post-translational processing. This segment is maximally conserved across all featured species and the VMA group, suggesting there may be mechanisms common to multiple species for control of PTHrP production in the post-translational phase. A long intron separates exons 5 and 6. While this intron is almost wholly conserved in the mouse, only a short segment is shared in the chicken, and no conservation is seen in the *Fugu* or *Xenopus* gene. Interestingly, the 5′ half of the intron features H3k27ac signal which peters out through the segment; this is correlated with patchy VMA consensus, indicating this may be a conserved regulatory mechanism.

Zone 3 contains exons 6–8 and comprises the bulk of the PTHrP protein product. Exon 6 codes 1–139 and is well conserved in both the VMA group and the species depicted. It has high conservation across the VMA's 28 species, suggesting it may code for a shared core function of PTHrP. Exons 7 and 8 and the intron between them are well conserved in both the rat and mouse, but not in vertebrates or the other species. Exon 7 codes an exon sandwiched between the 139 and 173 amino acid isoforms.

Zone 4 contains a long intron, the 5′ section of which is only conserved in the mouse, but the 3′ end of which is conserved in mouse, chicken and *Fugu*, but not in the VMA 28 species group. This precedes exon 9, which is highly conserved in mouse, chicken, *Fugu* and across the VMA species, but is absent from *Xenopus*.

The receptors for fish PTHrP and PTH were identified in zebrafish [111, 112]. In addition to identification of fish receptors equivalent to hPTH2R and hPTH1R, a third receptor was isolated and cloned [111]. The preferred ligand for this novel receptor (zPth3r) was hPTHrP(1–34). It was already known that the ligand for hPTH2R was tuberoinfundibular peptide 39 (TIP39), and its location was restricted to the brain [113], so it was inferred that fish Pthrp could bind to zPth1r and zPth3r [112].

Since PTHrP is produced by a number of mammalian epithelial tumors, including those of skin [114] and breast [115], the presence of the PTHrP antigen was examined in tumors from a number of lower vertebrates. Fish rarely develop true malignant tumors [116], but their neoplasms are usually benign and are delineated by their distinctive characteristic of progressive proliferation, which is uncoordinated with the surrounding tissue. A range of 51 neoplasms and tumors from bony and cartilaginous fish were examined for the presence of PTHrP, by immunohistochemistry using antisera to human PTHrP (1–14) [116]. The squamous carcinomata, renal adenocarcinoma and papillomata were positive for PTHrP, while the hepatocellular carcinomata, rhabdomyoma and myxoma were

Table 2 Results of immunohistochemistry performed with antisera to hPTHrP(1–14) on sections of fish tumors submitted to the Registry of Tumors in Lower Animals, George Washington University, Washington DC

Tumor type	Species	Skeletal type	Tumor Staining
Ameloblastic odontoma	*Tautagolabrus adspersus*	Bony	±
Cholangiocarcinoma	*Catostomus commersoni*	Bony	++
	Ameiurus nebulosus	Bony	+
	Ameiurus nebulosus	Bony	±
	Ameiurus nebulosus	Bony	±
	Ameiurus nebulosus	Bony	+/++
Chondroma	*Squalus acanthias*	Cartilaginous	–
Ependymoblastoma	*Oncorhynchus kisutch*	Bony	++
	Oncorhynchus kisutch	Bony	+/++
Epidermal papilloma	*Ameiurus melas*	Bony	++/+++
	Ameiurus melas	Bony	+++
	Genyonemus lineatus	Bony	++/+++
	Ameiurus nebulosus	Bony	++/+++
	Ameiurus nebulosus	Bony	++/+++
Fibroameloblastoma	*Oncorhynchus kisutch*	Bony	++
Fibroma	*Scomberomorus cavalla*	Bony	+
Fibrosarcoma	*Coregonus sp*	Bony	+
Ganglioneuroma	*Morone saxatilis*	Bony	++
Hepatocarcinoma	*Oncorhynchus mykiss*	Bony	++/+++
	Oncorhynchus mykiss	Bony	+++
Hepatocellular carcinoma	*Ameiurus nebulosus*	Bony	–
	Ameiurus nebulosus	Bony	±
	Ameiurus nebulosus	Bony	±
	Ameiurus nebulosus	Bony	–
	Ameiurus nebulosus	Bony	–
	Ameiurus nebulosus	Bony	–
	Ameiurus nebulosus	Bony	–
	Ameiurus nebulosus	Bony	–
	Ameiurus nebulosus	Bony	+
Leiomyoma	*Salmo trutta*	Bony	+
Lipoma	*Abramis brama*	Bony	+++
	Abramis brama	Bony	+++
Melanoma	*Ameiurus nebulosus*	Bony	++
Myxoma	*Mugil cephalus*	Bony	–
Nephroblastoma	*Oncorhynchus mykiss*	Bony	++
Osteoma	*Oncorhynchus tshawytscha*	Bony	++
Ovarian fibroma	*Micropterus sp*	Bony	++
Ovarian mucinous cystadenoma	*Micropterus salmonides*	Bony	–
	Micropterus salmonides	Bony	–

Table 2 continued

Tumor type	Species	Skeletal type	Tumor Staining
Papilloma (lip)	*Ameiurus nebulosus*	Bony	+/++
Pseudobranch pseudotumor	*Gadus macrocephalus*	Bony	++/+++
Renal adenocarcinoma	*Squalus acanthias*	Cartilaginous	++/+++
Rhabdomyoma (probable)	*Stizostedion vitreum vitreum*	Bony	–
Squamous carcinoma	*Ameiurus natalis*	Bony	+++
	Ameiurus nebulosus	Bony	+++
	Ameiurus nebulosus	Bony	++
	Ameiurus nebulosus	Bony	+
	Ameiurus nebulosus	Bony	+
	Ameiurus nebulosus	Bony	+
	Ameiurus nebulosus	Bony	±
Thyroid adenoma	*Negaprion brevirostris*	Cartilaginous	++
Thyroid hyperplasia versus adenoma	*Cyprinodon variegatus*	Bony	+

negative (Table 2, [116]). The finding of PTHrP in neoplasms of epithelial origin correlates well with the findings in mammalian tumors and indicates that PTHrP may be a marker of epithelial neoplasia in all vertebrates.

Amphibians and Birds

Amphibians were not only the first animals to have a distinct parathyroid gland but they were also the first to move out of water and onto land. The process of metamorphosis allows the aquatic larval stages to assume a tetrapod form and terrestrial physiology, which assists them to survive in a dry environment and without the support of water, which is relatively dense. Thus, once Pthrp had been identified in fish, this species was the next to investigate.

Immunohistochemistry was carried with antibodies raised to hPTHrP(1–14), and in situ hybridization used probes made to the chicken *PTHrP* gene sequence [117]. The animals (*Rana temporaria*) that were examined were those that were just emerging from water and so should have the tetrapod physiology that would allow them to control their calcium metabolism via their skeleton. The tissues that expressed the gene and possessed the antigen were skin, kidney, muscle, pituitary and the olfactory lobe of the brain. The lobes of pituitary did not have concordant results. The expression of the PTHrP mRNA was present in the pars intermedia only but the protein was present in the pars distalis, neural lobe, infundibular stalk and median eminence. This finding indicated that the pars intermedia was the site of production and translation of PTHrP mRNA but that the protein is transported to the pars distalis and then possibly by retrograde transport to the median eminence and infundibular stalk [117].

At the same time, the two forms of PTH1R were identified in *Xenopus laevis* [118] (Table 1). In adult frogs, one of the receptor isoforms (*xppr-a*) was found to be expressed in skin, lung, brain and small bowel while the other (*x-ppr-b*) was highest in lung, brain and heart. *xppr-a* expression was also detected in a small population of possible osteoblasts lining the perichondral and diaphyseal bone trabeculae. These two studies reinforce the view that there is a significant evolutionary pressure to conserve the localization of the ligand and the receptor, indicating that PTHrP functions are basic and fundamental in all vertebrates.

The gene sequence for *X. laevis pthrp* gene (Table 1) was deduced recently by an in silico study [119]. The gene structure is composed of at least ten exons, and the putative protein is 131 amino acids in length. There appears to be alternative splicing, and it was reported that the gene encodes for the largest 3′UTR region seen in vertebrates. The xPthrp protein has the ability to stimulate calcium uptake across frog skin [101], thus one of PTHrP roles in *Xenopus* is probably as a modulator of calcium metabolism.

Because of their egg-laying capacity, birds have to produce readily available calcium supply for eggshell production on a daily or seasonal basis. Most studies have been carried out on chickens because of their agricultural importance. Gay [120] demonstrated that the calcium metabolism in chickens was amplified compared to humans because of this very rapid need for calcium. PTH has been shown to elevate calcium levels in chickens [121] by reduced bone uptake [122] and its actions on the kidney. Thiede et al. [123] demonstrated chicken *PTHrP* expression in the avian oviduct and proposed that PTHrP might play a role as a modulator of the oviduct smooth muscle tension that is required during egg laying.

The gene for chicken *PTHrP* [124, 125] (Fig. 1) and the protein have been sequenced, and the protein is highly homologous to human PTHrP. Recent in silico studies predicted that chicken *PTHrP* gene consists of six exons, and the mature protein is 139 amino acids in length [119], although earlier work [125, 126] showing a five exon structure had predicted alternative splicing resulting in a protein of 139 amino acids or a protein of 141 amino acids (Fig. 1).

The *PTHrP* gene is expressed during skeletal histogenesis in the chick mandible [127]. Both PTHrP and the PTH1R were present in the chick embryo before the appearance of bone and were expressed by cells in the ectoderm, skeletal muscle, peripheral nerve and mesenchyme. Previously, chicken *PTHrP* and *PTH1R* gene expression had been demonstrated in the chicken

epiphyseal growth plate [128]. Both these studies support the role of PTHrP in the regulatory loop that is present in the chicken prehypertrophic and hypertrophic zone of the postnatal growth plate, thus indicating PTHrP involvement in bone formation in birds. Schermer and co-authors [125] found that *PTHrP* was expressed in adult chick brain, intestine, liver, skeletal muscle, heart, lung and gizzard. The expression of the gene in these tissues again reflects the situation in other vertebrates. The expression in the chick embryo suggested that at least part of the function of PTHrP in the chick is to influence cell growth and differentiation, reflecting its role in mammals.

Genescan dataset and a number of common tools such BLASTP and TBLASTN were used to examine the G protein-coupled receptors in the chicken genome [120]. It was hypothesized that the *cPTH1R* gene was a pseudogene as the fourth exon contained a stop codon. Given the role that PTH plays in avian calcium metabolism [121, 122], it seems very unlikely that cPTH1R is not functional. The fact that chickens do not have the PTH2R [129] or its ligand makes it unlikely that their PTH1R is inoperative. But the chickens do have *cPTH3R* which humans lack [119] (Table 1). This receptor was expressed predominantly in the hindgut, lung and cartilage of the chicken, while cPTH1R had the more ubiquitous expression profile that is seen in humans.

Conclusions

The recent sequencing of the whole lamprey genome (*Lethenteron japonicum*, [130]) is instructive. Agnathans (jawless fish) have a pivotal position in evolutionary history, having undergone two whole genome duplications when compared to invertebrates. They have a cartilaginous skeleton, and PTHrP has been localized in lamprey (*G. australis*) tissues using antisera raised against human PTHrP(1–34) [96]. This showed that the tissue localization was conserved among vertebrates and agnathans. Both *pth1r* and *pth2r* genes have been putatively identified in one of agnathan genome (*Petromyzon marinus*) [119] (Table 1).

Many questions remain unanswered. When did the gene duplication take place and which came first, PTH or PTHrP? Neither the PTH nor the PTHrP protein or gene has been identified in invertebrates. Interestingly, when hPTHrP (1–14) antiserum was used in immunohistochemistry on sections of an ascidian (*Pyura praeputialis*), the epithelia and the neural complex were positively stained [131]. This species is a sessile and solitary ascidian. Hull and co-authors [132] detected a PTH-like protein in snail brain but were unable to detect PTH immunoreactivity in hagfish, squid, cuttlefish or starfish. Thus, the presence of PTH or PTHrP in invertebrates is yet to be finally determined but two PTH receptors have been identified in *Ciona intestinalis* genome [133] (Table 1), indicating that these receptors pre-dated the formation of cartilage and bone.

Comparative endocrinology has established that PTHrP has a long evolutionary history, and the protein and its tissue localization are very highly conserved from fish to man. This strong evolutionary pressure to preserve PTHrP implies that PTHrP role(s) are basic and fundamental in all vertebrates. PTHrP clearly has roles in skeletal formation and cell proliferation and differentiation throughout evolution.

Disclosures

Conflict of Interest Janine A. Danks, Adam N. Freeman and T. John Martin declare that they have no conflict of interest.

Animal/Human Studies This article does not contain any studies with human or animal subjects performed by any of the authors.

References

1. Moseley JM, Kubota M, Diefenbach-Jagger H, Wettenhall RE, Kemp BE, Suva LJ, et al. Parathyroid hormone-related protein purified from a human lung cancer cell line. Proc Natl Acad Sci USA. 1987;84(14):5048–52.
2. Suva LJ, Winslow GA, Wettenhall RE, Hammonds RG, Moseley JM, Diefenbach-Jagger H, et al. A parathyroid hormone-related protein implicated in malignant hypercalcemia: cloning and expression. Science. 1987;237(4817):893–6.
3. Albright F. Case records of the Massachusetts general Hospital—case 39061. N Engl J Med. 1941;1941:789–96.
4. Berson SA, Yalow RS. Parathyroid hormone in plasma in adenomatous hyperparathyroidism, uremia, and bronchogenic carcinoma. Science. 1966;154(3751):907–9.
5. Lafferty FW. Pseudohyperparathyroidism. Medicine (Baltimore). 1966;45(3):247–60.
6. Roof BS, Carpenter B, Fink DJ, Gordan GS. Some thoughts on the nature of ectopic parathyroid hormones. Am J Med. 1971;50(5):686–91.
7. Riggs BL, Arnaud CD, Reynolds JC, Smith LH. Immunologic differentiation of primary hyperparathyroidism from hyperparathyroidism due to nonparathyroid cancer. J Clin Investig. 1971;50(10):2079–83.
8. Benson RC Jr, Riggs BL, Pickard BM, Arnaud CD. Immunoreactive forms of circulating parathyroid hormone in primary and ectopic hyperparathyroidism. J Clin Investig. 1974;54(1):175–81.
9. Melick RA, Martin TJ, Hicks JD. Parathyroid hormone production and malignancy. Br Med J. 1972;2(5807):204–5.
10. Powell D, Singer FR, Murray TM, Minkin C, Potts JT Jr. Nonparathyroid humoral hypercalcemia in patients with neoplastic diseases. N Engl J Med. 1973;289(4):176–81.
11. Martin TJ, Atkins D. Biochemical regulators of bone resorption and their significance in cancer. Essays Med Biochem. 1979;4:49–82.
12. Stewart AF, Horst R, Deftos LJ, Cadman EC, Lang R, Broadus AE. Biochemical evaluation of patients with cancer-associated hypercalcemia: evidence for humoral and nonhumoral groups. N Engl J Med. 1980;303(24):1377–83.
13. Rude RK, Sharp CF Jr, Fredericks RS, Oldham SB, Elbaum N, Link J, et al. Urinary and nephrogenous adenosine 3',5'-monophosphate in the hypercalcemia of malignancy. J Clin Endocrinol Metab. 1981;52(4):765–71.
14. Kukreja SC, Shemerdiak WP, Lad TE, Johnson PA. Elevated nephrogenous cyclic AMP with normal serum parathyroid

hormone levels in patients with lung cancer. J Clin Endocrinol Metab. 1980;51(1):167–9.
15. Atkins D, Hunt NH, Ingleton PM, Martin TJ. Rat osteogenic sarcoma cells: isolation and effects of hormones on the production of cyclic AMP and cyclic GMP. Endocrinology. 1977;101(2):555–61.
16. Partridge NC, Alcorn D, Michelangeli VP, Kemp BE, Ryan GB, Martin TJ. Functional properties of hormonally responsive cultured normal and malignant rat osteoblastic cells. Endocrinology. 1981;108(1):213–9.
17. Majeska RJ, Rodan SB, Rodan GA. Parathyroid hormone-responsive clonal cell lines from rat osteosarcoma. Endocrinology. 1980;107(5):1494–503.
18. Stewart AF, Insogna KL, Goltzman D, Broadus AE. Identification of adenylate cyclase-stimulating activity and cytochemical glucose-6-phosphate dehydrogenase-stimulating activity in extracts of tumors from patients with humoral hypercalcemia of malignancy. Proc Natl Acad Sci USA. 1983;80(5):1454–8.
19. Strewler GJ, Williams RD, Nissenson RA. Human renal carcinoma cells produce hypercalcemia in the nude mouse and a novel protein recognized by parathyroid hormone receptors. J Clin Investig. 1983;71(3):769–74.
20. Rodan SB, Insogna KL, Vignery AM, Stewart AF, Broadus AE, D'Souza SM, et al. Factors associated with humoral hypercalcemia of malignancy stimulate adenylate cyclase in osteoblastic cells. J Clin Investig. 1983;72(4):1511–5.
21. Goltzman D, Stewart AF, Broadus AE. Malignancy-associated hypercalcemia: evaluation with a cytochemical bioassay for parathyroid hormone. J Clin Endocrinol Metab. 1981;53(5): 899–904.
22. Stewart AF, Wu T, Goumas D, Burtis WJ, Broadus AE. N-terminal amino acid sequence of two novel tumor-derived adenylate cyclase-stimulating proteins: identification of parathyroid hormone-like and parathyroid hormone-unlike domains. Biochem Biophys Res Commun. 1987;146(2):672–8.
23. Strewler GJ, Stern PH, Jacobs JW, Eveloff J, Klein RF, Leung SC, et al. Parathyroid hormonelike protein from human renal carcinoma cells. Structural and functional homology with parathyroid hormone. J Clin Investig. 1987;80(6):1803–7.
24. Mangin M, Ikeda K, Broadus AE. Structure of the mouse gene encoding parathyroid hormone-related peptide. Gene. 1990;95(2): 195–202.
25. Kemp BE, Moseley JM, Rodda CP, Ebeling PR, Wettenhall RE, Stapleton D, et al. Parathyroid hormone-related protein of malignancy: active synthetic fragments. Science. 1987; 238(4833):1568–70.
26. Moseley JM, Gillespie MT. Parathyroid hormone-related protein. Crit Rev Clin Lab Sci. 1995;32(3):299–343.
27. Moseley JM, Hayman JA, Danks JA, Alcorn D, Grill V, Southby J, et al. Immunohistochemical detection of parathyroid hormone-related protein in human fetal epithelia. J Clin Endocr Metab. 1991;73(3):478–84.
28. Karaplis AC, Luz A, Glowacki J, Bronson RT, Tybulewicz VL, Kronenberg HM, et al. Lethal skeletal dysplasia from targeted disruption of the parathyroid hormone-related peptide gene. Genes Dev. 1994;8(3):277–89.
29. Amizuka N, Warshawsky H, Henderson JE, Goltzman D, Karaplis AC. Parathyroid hormone-related peptide-depleted mice show abnormal epiphyseal cartilage development and altered endochondral bone formation. J Cell Biol. 1994;126(6):1611–23.
30. Lanske B, Karaplis AC, Lee K, Luz A, Vortkamp A, Pirro A, et al. PTH/PTHrP receptor in early development and Indian hedgehog-regulated bone growth. Science. 1996;273(5275): 663–6.
31. Vortkamp A, Lee K, Lanske B, Segre GV, Kronenberg HM, Tabin CJ. Regulation of rate of cartilage differentiation by Indian hedgehog and PTH-related protein. Science. 1996; 273(5275):613–22.
32. Amizuka N, Karaplis AC, Henderson JE, Warshawsky H, Lipman ML, Matsuki Y, et al. Haploinsufficiency of parathyroid hormone-related peptide (PTHrP) results in abnormal postnatal bone development. Dev Biol. 1996;175(1):166–76.
33. Miao D, He B, Jiang Y, Kobayashi T, Soroceanu MA, Zhao J, et al. Osteoblast-derived PTHrP is a potent endogenous bone anabolic agent that modifies the therapeutic efficacy of administered PTH 1-34. J Clin Investig. 2005;115(9):2402–11.
34. Charbon GA. A rapid and selective vasodialtor effect of parathyroid hormone. Eur J Pharmacol. 1968;3(3):275–8.
35. Charbon GA, Hulstaert PF. Augmentation of arterial hepatic and renal flow by extracted and synthetic parathyroid hormone. Endocrinology. 1974;95(2):621–6.
36. Wang HH, Drugge ED, Yen YC, Blumenthal MR, Pang PK. Effects of synthetic parathyroid hormone on hemodynamics and regional blood flows. Eur J Pharmacol. 1984;97(3–4):209–15.
37. Mok LL, Nickols GA, Thompson JC, Cooper CW. Parathyroid hormone as a smooth muscle relaxant. Endocr Rev. 1989;10(4): 420–36.
38. Pirola CJ, Wang HM, Kamyar A, Wu S, Enomoto H, Sharifi B, et al. Angiotensin II regulates parathyroid hormone-related protein expression in cultured rat aortic smooth muscle cells through transcriptional and post-transcriptional mechanisms. J Biol Chem. 1993;268(3):1987–94.
39. Massfelder T, Helwig JJ, Stewart AF. Parathyroid hormone-related protein as a cardiovascular regulatory peptide. Endocrinology. 1996;137(8):3151–3.
40. Winquist RJ, Baskin EP, Vlasuk GP. Synthetic tumor-derived human hypercalcemic factor exhibits parathyroid hormone-like vasorelaxation in renal arteries. Biochem Biophys Res Commun. 1987;149(1):227–32.
41. Shew RL, Yee JA, Kliewer DB, Keflemariam YJ, McNeill DL. Parathyroid hormone-related protein inhibits stimulated uterine contraction in vitro. J Bone Miner Res. 1991;6(9):955–9.
42. Philbrick WM, Wysolmerski JJ, Galbraith S, Holt E, Orloff JJ, Yang KH, et al. Defining the roles of parathyroid hormone-related protein in normal physiology. Physiol Rev. 1996;76(1):127–73.
43. Martin TJ, Moseley JM, Williams ED. Parathyroid hormone-related protein: hormone and cytokine. J Endocrinol. 1997; 154(Suppl):S23–37.
44. Aarts MM, Rix A, Guo J, Bringhurst R, Henderson JE. The nucleolar targeting signal (NTS) of parathyroid hormone related protein mediates endocytosis and nucleolar translocation. J Bone Miner Res. 1999;14(9):1493–503.
45. Henderson JE, Amizuka N, Warshawsky H, Biasotto D, Lanske BM, Goltzman D, et al. Nucleolar localization of parathyroid hormone-related peptide enhances survival of chondrocytes under conditions that promote apoptotic cell death. Mol Cell Biol. 1995;15(8):4064–75.
46. Okano K, Pirola CJ, Wang HM, Forrester JS, Fagin JA, Clemens TL. Involvement of cell cycle and mitogen-activated pathways in induction of parathyroid hormone-related protein gene expression in rat aortic smooth muscle cells. Endocrinology. 1995;136(4):1782–9.
47. Lam MH, Olsen SL, Rankin WA, Ho PW, Martin TJ, Gillespie MT, et al. PTHrP and cell division: expression and localization of PTHrP in a keratinocyte cell line (HaCaT) during the cell cycle. J Cell Physiol. 1997;173(3):433–46.
48. Nguyen M, He B, Karaplis A. Nuclear forms of parathyroid hormone-related peptide are translated from non-AUG start sites downstream from the initiator methionine. Endocrinology. 2001;142(2):694–703.
49. Massfelder T, Dann P, Wu TL, Vasavada R, Helwig JJ, Stewart AF. Opposing mitogenic and anti-mitogenic actions of parathyroid

hormone-related protein in vascular smooth muscle cells: a critical role for nuclear targeting. Proc Natl Acad Sci USA. 1997;94(25): 13630–5.
50. Jans DA. The regulation of protein transport to the nucleus by phosphorylation. Biochem J. 1995;311(Pt 3):705–16.
51. Lam MH, Thomas RJ, Loveland KL, Schilders S, Gu M, Martin TJ, et al. Nuclear transport of parathyroid hormone (PTH)-related protein is dependent on microtubules. Mol Endocrinol. 2002;16(2):390–401.
52. Cingolani G, Bednenko J, Gillespie MT, Gerace L. Molecular basis for the recognition of a nonclassical nuclear localization signal by importin beta. Mol Cell. 2002;10(6):1345–53.
53. Amizuka N, Fukushi-Irie M, Sasaki T, Oda K, Ozawa H. Inefficient function of the signal sequence of PTHrP for targeting into the secretory pathway. Biochem Biophys Res Commun. 2000;273(2):621–9.
54. Lam MH, House CM, Tiganis T, Mitchelhill KI, Sarcevic B, Cures A, et al. Phosphorylation at the cyclin-dependent kinases site (Thr85) of parathyroid hormone-related protein negatively regulates its nuclear localization. J Biol Chem. 1999;274(26): 18559–66.
55. Jans DA, Briggs LJ, Gustin SE, Jans P, Ford S, Young IG. The cytokine interleukin-5 (IL-5) effects cotransport of its receptor subunits to the nucleus in vitro. FEBS Lett. 1997;410(2–3):368–72.
56. Liu Y, Berendsen AD, Jia S, Lotinun S, Baron R, Ferrara N, et al. Intracellular VEGF regulates the balance between osteoblast and adipocyte differentiation. J Clin Investig. 2012;122(9): 3101–13.
57. Watson PH, Fraher LJ, Natale BV, Kisiel M, Hendy GN, Hodsman AB. Nuclear localization of the type 1 parathyroid hormone/parathyroid hormone-related peptide receptor in MC3T3-E1 cells: association with serum-induced cell proliferation. Bone. 2000;26(3):221–5.
58. Watson PH, Fraher LJ, Hendy GN, Chung UI, Kisiel M, Natale BV, et al. Nuclear localization of the type 1 PTH/PTHrP receptor in rat tissues. J Bone Miner Res. 2000;15(6):1033–44.
59. Juppner H, Abou-Samra AB, Freeman M, Kong XF, Schipani E, Richards J, et al. A G protein-linked receptor for parathyroid hormone and parathyroid hormone-related peptide. Science. 1991;254(5034):1024–6.
60. Rodda CP, Kubota M, Heath JA, Ebeling PR, Moseley JM, Care AD, et al. Evidence for a novel parathyroid hormone-related protein in fetal lamb parathyroid glands and sheep placenta: comparisons with a similar protein implicated in humoral hypercalcaemia of malignancy. J Endocrinol. 1988;117(2):261–71.
61. Abbas SK, Pickard DW, Rodda CP, Heath JA, Hammonds RG, Wood WI, et al. Stimulation of ovine placental calcium transport by purified natural and recombinant parathyroid hormone-related protein (PTHrP) preparations. Q J Exp Physiol. 1989;74(4): 549–52.
62. Kovacs CS, Lanske B, Hunzelman JL, Guo J, Karaplis AC, Kronenberg HM. Parathyroid hormone-related peptide (PTHrP) regulates fetal–placental calcium transport through a receptor distinct from the PTH/PTHrP receptor. Proc Natl Acad Sci USA. 1996;93(26):15233–8.
63. Fenton AJ, Martin TJ, Nicholson GC. Long-term culture of disaggregated rat osteoclasts: inhibition of bone resorption and reduction of osteoclast-like cell number by calcitonin and PTHrP[107–139]. J Cell Physiol. 1993;155(1):1–7.
64. Cornish J, Callon KE, Nicholson GC, Reid IR. Parathyroid hormone-related protein-(107–139) inhibits bone resorption in vivo. Endocrinology. 1997;138(3):1299–304.
65. Cornish J, Callon KE, Lin C, Xiao C, Moseley JM, Reid IR. Stimulation of osteoblast proliferation by C-terminal fragments of parathyroid hormone-related protein. J Bone Miner Res. 1999;14(6):915–22.
66. de Gortazar AR, Alonso V, Alvarez-Arroyo MV, Esbrit P. Transient exposure to PTHrP (107–139) exerts anabolic effects through vascular endothelial growth factor receptor 2 in human osteoblastic cells in vitro. Calcif Tissue Int. 2006;79(5):360–9.
67. Alonso V, de Gortazar AR, Ardura JA, Andrade-Zapata I, Alvarez-Arroyo MV, Esbrit P. Parathyroid hormone-related protein (107–139) increases human osteoblastic cell survival by activation of vascular endothelial growth factor receptor-2. J Cell Physiol. 2008;217(3):717–27.
68. Portal-Nunez S, Lozano D, de Castro LF, de Gortazar AR, Nogues X, Esbrit P. Alterations of the Wnt/beta-catenin pathway and its target genes for the N- and C-terminal domains of parathyroid hormone-related protein in bone from diabetic mice. FEBS Lett. 2010;584(14):3095–100.
69. Valin A, Guillen C, Esbrit P. C-terminal parathyroid hormone-related protein (PTHrP) (107–139) stimulates intracellular Ca(2+) through a receptor different from the type 1 PTH/PTHrP receptor in osteoblastic osteosarcoma UMR 106 cells. Endocrinology. 2001;142(7):2752–9.
70. Divieti P, Inomata N, Chapin K, Singh R, Juppner H, Bringhurst FR. Receptors for the carboxyl-terminal region of pth(1–84) are highly expressed in osteocytic cells. Endocrinology. 2001;142(2): 916–25.
71. Miao D, Su H, He B, Gao J, Xia Q, Zhu M, et al. Severe growth retardation and early lethality in mice lacking the nuclear localization sequence and C-terminus of PTH-related protein. Proc Natl Acad Sci USA. 2008;105(51):20309–14.
72. Toribio RE, Brown HA, Novince CM, Marlow B, Hernon K, Lanigan LG, et al. The midregion, nuclear localization sequence, and C terminus of PTHrP regulate skeletal development, hematopoiesis, and survival in mice. FASEB J. 2010; 24(6):1947–57.
73. Burtis WJ, Fodero JP, Gaich G, Debeyssey M, Stewart AF. Preliminary characterization of circulating amino- and carboxy-terminal fragments of parathyroid hormone-related peptide in humoral hypercalcemia of malignancy. J Clin Endocrinol Metab. 1992;75(4):1110–4.
74. Bruns ME, Ferguson JE 2nd, Bruns DE, Burton DW, Brandt DW, Juppner H, et al. Expression of parathyroid hormone-related peptide and its receptor messenger ribonucleic acid in human amnion and chorion-decidua: implications for secretion and function. Am J Obstet Gynecol. 1995;173(3 Pt 1):739–46.
75. Orloff JJ, Reddy D, de Papp AE, Yang KH, Soifer NE, Stewart AF. Parathyroid hormone-related protein as a prohormone: posttranslational processing and receptor interactions. Endocr Rev. 1994;15(1):40–60.
76. Fiaschi-Taesch NM, Stewart AF. Minireview: parathyroid hormone-related protein as an intracrine factor–trafficking mechanisms and functional consequences. Endocrinology. 2003;144(2): 407–11.
77. Mangin M, Ikeda K, Dreyer BE, Milstone L, Broadus AE. Two distinct tumor-derived, parathyroid hormone-like peptides result from alternative ribonucleic acid splicing. Mol Endocrinol. 1988;2(11):1049–55.
78. Thiede MA, Strewler GJ, Nissenson RA, Rosenblatt M, Rodan GA. Human renal carcinoma expresses two messages encoding a parathyroid hormone-like peptide: evidence for the alternative splicing of a single-copy gene. Proc Natl Acad Sci USA. 1988;85(13):4605–9.
79. Yasuda T, Banville D, Hendy GN, Goltzman D. Characterization of the human parathyroid hormone-like peptide gene. Functional and evolutionary aspects. J Biol Chem. 1989; 264(13):7720–5.
80. Mangin M, Ikeda K, Dreyer BE, Broadus AE. Isolation and characterization of the human parathyroid hormone-like peptide gene. Proc Natl Acad Sci USA. 1989;86(7):2408–12.

81. Suva LJ, Mather KA, Gillespie MT, Webb GC, Ng KW, Winslow GA, et al. Structure of the 5' flanking region of the gene encoding human parathyroid-hormone-related protein (PTHrP). Gene. 1989;77(1):95–105.
82. Comings D. Evidence for ancient tetraploidy and conservation of linkage groups in mammalian chromosomes. Nature. 1972;238:455–7.
83. Danks JA, Trivett MK. Endocrinology and evolution: lessons from comparative endocrinology. In: Wass JAH, editor. Oxford textbook of endocrinology and diabetes. 1 ed. Oxford: Oxford University Press; 2002. p. 9–13.
84. Vasavada RC, Wysolmerski JJ, Broadus AE, Philbrick WM. Identification and characterization of a GC-rich promoter of the human parathyroid hormone-related peptide gene. Mol Endocrinol. 1993;7(2):273–82.
85. Zajac JD, Callaghan J, Eldridge C, Diefenbach-Jagger H, Suva LJ, Hudson P, et al. Production of parathyroid hormone-related protein by a rat parathyroid cell line. Mol Cell Endocrinol. 1989;67(1):107–12.
86. Ikeda K, Mangin M, Dreyer BE, Webb AC, Posillico JT, Stewart AF, et al. Identification of transcripts encoding a parathyroid hormone-like peptide in messenger RNAs from a variety of human and animal tumors associated with humoral hypercalcemia of malignancy. J Clin Investig. 1988;81(6):2010–4.
87. Butkus A, Roche PJ, Fernley RT, Haralambidis J, Penschow JD, Ryan GB, et al. Purification and cloning of a corpuscles of Stannius protein from *Anguilla australis*. Mol Cell Endocrinol. 1987;54(2–3):123–33.
88. Chang ACM, Janosi J, Hulsbeek M, Dejong D, Jeffrey KJ, Noble JR, et al. A novel human cDNA highly homologous to the fish hormone stanniocalcin. Mol Cell Endocrinol. 1995;112(2):241–7.
89. Ingleton PM, Danks JA. Parathyroid hormone-related protein in the saccus vasculosus of fishes. In: Dacke C, Danks J, Caple I, Flik G, editors. The comparative endocrinology of calcium regulation. Bristol: Journal of Endocrinology Ltd; 1996. p. 103–5.
90. Danks JA, Hubbard PC, Balment RJ, Ingleton PM, Martin TJ. Parathyroid hormone-related protein localization in tissues of freshwater and salt-acclimated flounder. J Bone Miner Res. 1996;11:435.
91. Danks JA, Devlin AJ, Ho PMW, Diefenbachjagger H, Power DM, Canario A, et al. Parathyroid hormone-related protein is a factor in normal fish pituitary. Gen Comp Endocrinol. 1993;92(2):201–12.
92. Parsons JA, Gray, D, Rafferty B, Zanelli JM. Evidence for a hypercalcaemic factor in the fish pituitary immunologically related to mammalian parathyroid hormone. In: Copp DH, Talmage RV, editors. Endocrinology of calcium metabolism. Proceedings of the 6th parathyroid conference, Vancouver, Canada, June 12–17, 1977. Amsterdam: Excerpta Medica; 1979. p. 111–4.
93. Kaneko T, Pang PK. Immunocytochemical detection of parathyroid hormone-like substance in the goldfish brain and pituitary gland. Gen Comp Endocrinol. 1987;68(1):147–52.
94. Fraser RA, Kaneko T, Pang PK, Harvey S. Hypo- and hypercalcemic peptides in fish pituitary glands. Am J Physiol. 1991;260(3 Pt 2):R622–6.
95. Trivett MK, Officer RA, Clement JG, Walker TI, Joss JM, Ingleton PM, et al. Parathyroid hormone-related protein (PTHrP) in cartilaginous and bony fish tissues. J Exp Zool. 1999;284(5):541–8.
96. Trivett MK, Potter IC, Power G, Zhou H, Macmillan DL, Martin TJ, et al. Parathyroid hormone-related protein production in the lamprey *Geotria australis*: developmental and evolutionary perspectives. Dev Genes Evol. 2005;215(11):553–63.
97. Trivett MK, Walker TI, Clement JG, Ho PM, Martin TJ, Danks JA. Effects of water temperature and salinity on parathyroid hormone-related protein in the circulation and tissues of elasmobranchs. Comp Biochem Physiol B: Biochem Mol Biol. 2001;129(2–3):327–36.
98. Danks JA, Trivett MK, Power DM, Canario AV, Martin TJ, Ingleton PM. Parathyroid hormone-related protein in lower vertebrates. Clin Exp Pharmacol Physiol. 1998;25(9):750–2.
99. Danks JA, Ho PM, Notini AJ, Katsis F, Hoffmann P, Kemp BE, et al. Identification of a parathyroid hormone in the fish *Fugu rubripes*. J Bone Miner Res. 2003;18(7):1326–31.
100. Canario AVM, Rotllant J, Fuentes J, Guerreiro PM, Teodosio HR, Power DM, et al. Novel bioactive parathyroid hormone and related peptides in teleost fish. FEBS Lett. 2006;580(1):291–9.
101. Pinheiro PLC, Cardoso JCR, Gomes AS, Fuentes J, Power DM, Canario AVM. Gene structure, transcripts and calciotropic effects of the PTH family of peptides in *Xenopus* and chicken. BMC Evol Biol. 2010;10:373. doi:10.1186/1471-2148-10-373.
102. Ohno S. Evolution by gene duplication. Berlin: Springer; 1970.
103. Lynch M, Force A. The probability of duplicate gene preservation by subfunctionalization. Genetics. 2000;154(1):459–73.
104. Liu Y, Ibrahim AS, Tay BH, Richardson SJ, Bell J, Walker TI, et al. Parathyroid hormone gene family in a cartilaginous fish, the elephant shark (*Callorhinchus milii*). J Bone Miner Res. 2010;25(12):2613–23.
105. Venkatesh B, Kirkness EF, Loh YH, Halpern AL, Lee AP, Johnson J, et al. Survey sequencing and comparative analysis of the elephant shark (*Callorhinchus milii*) genome. PLoS Biol. 2007;5(4):e101.
106. Yan YL, Bhattacharya P, He XJ, Ponugoti B, Marquardt B, Layman J, et al. Duplicated zebrafish co-orthologs of parathyroid hormone-related peptide (PTHrP, Pthlh) play different roles in craniofacial skeletogenesis. J Endocrinol. 2012;214(3):421–35.
107. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, et al. The human genome browser at UCSC. Genome Res. 2002;12(6):996–1006.
108. Siepel A, Pollard KS, Haussler D. New methods for detecting lineage-specific selection. Res Comput Mol Biol Lect Notes Comput Sci. 2006;3909:190–205.
109. Murphy WJ, Eizirik E, O'Brien SJ, Madsen O, Scally M, Douady CJ, et al. Resolution of the early placental mammal radiation using Bayesian phylogenetics. Science. 2001;294(5550):2348–51.
110. Rosenbloom KR, Sloan CA, Malladi VS, Dreszer TR, Learned K, Kirkup VM, et al. ENCODE data in the UCSC genome browser: year 5 update. Nucleic Acids Res. 2013;41(Database issue):D56–63.
111. Rubin DA, Hellman P, Zon LI, Lobb CJ, Bergwitz C, Juppner H. A G protein-coupled receptor from zebrafish is activated by human parathyroid hormone and not by human or teleost parathyroid hormone-related peptide. Implications for the evolutionary conservation of calcium-regulating peptide hormones. J Biol Chem. 1999;274(33):23035–42.
112. Rubin DA, Juppner H. Zebrafish express the common parathyroid hormone/parathyroid hormone-related peptide receptor (PTH1R) and a novel receptor (PTH3R) that is preferentially activated by mammalian and fugufish parathyroid hormone-related peptide. J Biol Chem. 1999;274(40):28185–90.
113. Usdin TB, Hoare SR, Wang T, Mezey E, Kowalak JA. TIP39: a new neuropeptide and PTH2-receptor agonist from hypothalamus. Nat Neurosci. 1999;2(11):941–3.
114. Danks JA, Ebeling PR, Hayman J, Chou ST, Moseley JM, Dunlop J, et al. Parathyroid hormone-related protein—immunohistochemical localization in cancers and in normal skin. J Bone Miner Res. 1989;4(2):273–8.

115. Southby J, Kissin MW, Danks JA, Hayman JA, Moseley JM, Henderson MA, et al. Immunohistochemical localization of parathyroid hormone-related protein in human breast-cancer. Cancer Res. 1990;50(23):7710–6.
116. Trivett MK, Harshbarger JC, Martin TJ, Danks JA. Is parathyroid hormone-related protein a marker of neoplasia in all vertebrates? J Bone Miner Res. 2000;15:S321.
117. Danks JA, McHale JC, Martin TJ, Ingleton PM. Parathyroid hormone-related protein in tissues of the emerging frog (*Rana temporaria*): immunohistochemistry and in situ hybridisation. J Anat. 1997;190(Pt 2):229–38.
118. Bergwitz C, Klein P, Kohno H, Forman SA, Lee K, Rubin D, et al. Identification functional characterization, and developmental expression of two nonallelic parathyroid hormone (PTH)/PTH-related peptide receptor isoforms in *Xenopus laevis* (daudin). Endocrinology. 1998;139(2):723–32.
119. Pinheiro PLC, Cardoso JCR, Power DM, Canario AVM. Functional characterization and evolution of PTH/PTHrP receptors: insights from the chicken. BMC Evol Biol. 2012;12:110.
120. Gay CV. Avian bone resorption at a cellular level. CRC Crit Rev Poult Biol. 1988;1:197–210.
121. Kenny AD, Dacke CG. Hypercalcemic response to parathyroid-hormone in Japanese-quail. J Endocrinol. 1974;62(1):15–23.
122. Dacke CG, Shaw AJ. Studies of the rapid effects of parathyroid-hormone and prostaglandins on Ca-45 uptake into chick and rat bone invivo. J Endocrinol. 1987;115(3):369–77.
123. Thiede MA, Harm SC, Mckee RL, Grasser WA, Duong LT, Leach RM. Expression of the parathyroid hormone-related protein gene in the avian oviduct—potential role as a local modulator of vascular smooth-muscle tension and shell gland motility during the egg-laying cycle. Endocrinology. 1991;129(4):1958–66.
124. Khosla S, Demay M, Pines M, Hurwitz S, Potts JT Jr, Kronenberg HM. Nucleotide sequence of cloned cDNAs encoding chicken preproparathyroid hormone. J Bone Miner Res. 1988; 3(6):689–98.
125. Schermer DT, Chan SDH, Bruce R, Nissenson RA, Wood WI, Strewler GJ. Chicken parathyroid hormone-related protein and its expression during embryologic development. J Bone Miner Res. 1991;6(2):149–55.
126. Thiede MA, Rutledge SJ. Nucleotide-sequence of a parathyroid hormone-related peptide expressed by the 10 day chicken-embryo. Nucleic Acids Res. 1990;18(10):3062.
127. Zhao Q, Brauer PR, Xiao L, McGuire MH, Yee JA. Expression of parathyroid hormone-related peptide (PthrP) and its receptor (PTH1R) during the histogenesis of cartilage and bone in the chicken mandibular process. J Anat. 2002;201(2):137–51.
128. Medill NJ, Praul CA, Ford BC, Leach RM. Parathyroid hormone-related peptide expression in the epiphyseal growth plate of the juvenile chicken: evidence for the origin of the parathyroid hormone-related peptide found in the epiphyseal growth plate. J Cell Biochem. 2001;80(4):504–11.
129. Bhattacharya P, Yan YL, Postlethwait J, Rubin DA. Evolution of the vertebrate pth2 (tip39) gene family and the regulation of PTH type 2 receptor (pth2r) and its endogenous ligand pth2 by hedgehog signaling in zebrafish development. J Endocrinol. 2011;211(2):187–200.
130. Mehta TK, Ravi V, Yamasaki S, Lee AP, Lian MM, Tay BH, et al. Evidence for at least six Hox clusters in the Japanese lamprey (*Lethenteron japonicum*). Proc Natl Acad Sci USA. 2013;110(40):16044–9.
131. Danks JA, D'Souza DG, Gunn HJ, Milley KM, Richardson SJ. Evolution of the parathyroid hormone family and skeletal formation pathways. Gen Comp Endocrinol. 2011;170(1):79–91.
132. Hull K, Marler R, Harvey S. Neural calcitropic peptides: immunoreactive characterization in fish and invertebrates. Neurosci Lett. 2006;404(1–2):15–9.
133. Kamesh N, Aradhyam GK, Manoj N. The repertoire of G protein-coupled receptors in the sea squirt *Ciona intestinalis*. BMC Evol Biol. 2008;8:129.