Inorganic pyrophosphate generation and disposition in pathophysiology

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Terkeltaub, Robert A. Inorganic pyrophosphate generation and disposition in pathophysiology. Am J Physiol Cell Physiol 281: C1–C11, 2001.—Inorganic pyrophosphate (PPi) regulates certain intracellular functions and extracellular crystal deposition. PPi is produced, degraded, and transported by specialized mechanisms. Moreover, dysregulated cellular PPi production, degradation, and transport all have been associated with disease, and PPi appears to directly mediate specific disease manifestations. In addition, natural and synthetic analogs of PPi are in use or currently under evaluation as prophylactic agents or therapies for disease. This review summarizes recent developments in the understanding of how PPi is made and disposed of by cells and assesses the body of evidence for potentially significant physiological functions of intracellular PPi, in higher organisms. Major topics addressed are recent lines of molecular evidence that directly link decreased and increased extracellular PPi levels with diseases in which connective tissue matrix calcification is disordered. To illustrate in depth the effects of disordered PPi metabolism, this review weighs the roles in matrix calcification of the transmembrane protein ANK, which regulates intracellular to extracellular movement of PPi, and the PPi-generating phosphodiesterase nucleotide pyrophosphatase family isoenzyme plasma cell membrane glycoprotein-1 (PC-1).

PC-1; ANK; alkaline phosphatase; inorganic pyrophosphatase; chondrocalcinosi s; hydroxyapatite

INORGANIC PYROPHOSPHATE (PPi) is comprised of two molecules of inorganic phosphate (Pi) joined by a hydrolyzable high energy ester bond (see Fig. 1). Natural compounds similar in structure to PPi include the crystallization inhibitor phosphocitrate (15, 16, 76) (see Fig. 1), which is a synthetic product of citrate and ATP, and is enriched in mitochondria. Usages of phosphocitrate and phosphocitrate analogs as crystallization inhibitors, and to block hydroxyapatite-induced cell stimulation (77), have been reviewed in detail elsewhere (15). However, some of the described effects of phosphocitrate on crystallization may be mediated in part by citrate effects (111).

A broadly employed class of synthetic, therapeutic compounds, the bisphosphonates (Fig. 1), have a carbon instead of an oxygen molecule separating two phosphates, which allows these drugs to serve in part as nonhydrolyzable PPi analogs. The reader is referred to other sources for appropriately detailed discussion of bisphosphonates, including their therapeutic uses and the potential of some bisphosphonates to interfere with protein isoprenylation and/or to modulate other intracellular biochemical pathways (28, 94, 101).

Understanding how PPi is generated and disposed of is pertinent to discussion of how PPi functions. Recently, a PPi synthase was cloned in Rhodospirillum rubrum (6). However, PPi is not known to be synthesized de novo in mammalian cells, which generate PPi either as a metabolic byproduct of numerous biochemical and biosynthetic reactions (91) or directly by pyrophosphohydrolysis of the phosphodiesterase I bond in
ppi can become compartmentalized in cells and subcellular organelles (50–52). Correspondingly, evidence exists for compartmentalized ppi in mitochondria and extracellular ppi (79, 123, 124) (Table 1). Recent comparative analyses of primary structures have revealed that the agents are aminobisphosphonates or nonaminobisphosphonates and modifies their functional properties, as reviewed elsewhere.

Though pp does not passively cross cell membranes, pp can become compartmentalized in cells and subcellular organelles (50–52). Correspondingly, evidence exists for active and facilitated pp transport mechanisms (66), including a saturable pathway for uptake of pp by several microorganisms that is sensitive to orthophosphate (122), and in mammalian cells, a mitochondrial membrane pp-nucleotide transport system that exchanges pp for ADP (68). It is not known whether the latter transport system exists in the plasma membrane. However, recent studies have elucidated a mechanism for pp channeling to the cell exterior that is mediated by the multiple-pass membrane protein ANK in mammalian cells (43). This pathway markedly influences both intracellular and extracellular pp concentrations (43).

Inorganic pyrophosphatases include membrane-binding and proton-pumping forms identified in lower organisms and soluble forms that have been well characterized in higher organisms (7, 8, 26, 27, 59, 81, 92, 110, 113, 126, 127). Importantly, cytosolic pp concentration in cells of higher organisms is clearly regulated and under the influence of soluble inorganic pyrophosphatase activity that is effective at neutral pH (EC 3.6.1.1) (Table 1) (8, 113). Inorganic pyrophosphatase activity levels vary between different mammalian tissues, likely attributable to tissue-specific regulation of gene expression (27, 126). A particularly high specific activity of inorganic pyrophosphatase activity is present in retinal rod outer segments (126). pp regulates guanylate cyclase, and inorganic pyrophosphatase activity may need to be enriched in vertebrate photoreceptor cells because of the high levels of cyclic nucleotide metabolism described there (40). Polyamines also regulate pp concentrations through their ability to form a complex with pp, that serves as a substrate for inorganic pyrophosphatase activity (60).

Members of the alkaline phosphatase (AP) family of ectoenzymes, which catalyze pp degradation at an alkaline pH optimum, exert major effects on extracellular pp concentrations (79, 123, 124) (Table 1). Recent comparative analyses of primary structures have revealed that APs and the PDNP family of NTPPPH metalloenzymes share certain features that mediate enzyme activity (32, 34).

Osteoclast tartrate-resistant acid phosphatase and certain other acid phosphatases also have efficient pyrophosphatase activity (61, 70). However, pp-degrading activity is not a universal activity of acid phosphatases (84). Moreover, the extent to which acid phosphatases physiologically and pathologically regulate pp metabolism in bone, prostate, and other tissues remains to be directly investigated.

PPi metabolism at the cellular level has been studied most extensively in cultured hepatocytes (23) and chondrocytes (47, 51, 95–97, 105, 106). It appears that a substantial fraction of intracellular pp is generated in the mitochondria, and intracellular and extracellular pp concentrations are both under the regulation of mitochondrial energy metabolism (23, 49). Soluble F1 from mitochondria appears to be involved in a complete catalytic cycle employing P, that results in the spontaneous synthesis of ATP and pp (121). Intramitochondrial pp is also under the control of calcium, which inhibits mitochondrial matrix inorganic pyrophosphatase activity (22, 24). Mitochondrially derived ATP has been established to be a major substrate for NTPPPH-mediated generation of both intracellular and extracellular pp by chondrocytes (49). However,

<table>
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<th>Table 1. Specialized extramitochondrial regulators of pp metabolism currently recognized in mammalian cells</th>
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<td><strong>Regulator</strong></td>
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<td><strong>Enzymes with inorganic pyrophosphatase activity</strong></td>
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*Also see Table 2. See text for definitions.
as a primordial "high energy" compound, and PP\textsubscript{i} (or donor in protein phosphorylation in not only yeast ATP-derived PP\textsubscript{i} also can function as a phosphate function in energy transfer reactions in yeast, where attention (33, 68) for discussions of the ability of PP\textsubscript{i} to overviews of PP\textsubscript{i} in mitochondrial metabolism merit mitochondria from transferrin (20). Certain previous between transferrin and ferritin and iron delivery to nase, and phosphofructokinase (PFK) have been found
[glucokinase, pyruvate kinase, phosphoglycerate ki-
attenuated (17). The PP\textsubscript{i}-utilizing enzymes involved
polyphosphates) is able to substitute for ATP under
certain circumstances, such as in glycolysis-related
putative intracellular functions of PP\textsubscript{i}

Extracellular PP\textsubscript{i} has been extensively studied in a pathophysiological context in mammals with respect to effects on crystallization (as discussed below). There has been less attention given to cell physiological functional roles of PP\textsubscript{i} in higher organisms, in part, because certain PP\textsubscript{i} functions related to energy metabolism identified in lower organisms appear to be limited to prokaryotes. In these organisms, PP\textsubscript{i} (like ATP) serves as a primordial “high energy” compound, and PP\textsubscript{i} (or polyphosphates) is able to substitute for ATP under certain circumstances, such as in glycolysis-related reactions in harsh environments where respiration is attenuated (17). The PP\textsubscript{i}-utilizing enzymes involved [glucokinase, pyruvate kinase, phosphoglycerate kinase, and phosphofructokinase (PFK)] have been found in anaerobic bacteria and proteozoa organisms, and in the case of PFKs, PP\textsubscript{i} dependency appears to be a late-evolving (and retained) adaptation (75). The ability of PP\textsubscript{i}, like other phosphate esters, to serve as an intracellular biochemical intermediate may be underestimated in higher organisms (17). For example, at physiological concentrations, PP\textsubscript{i} can interact with and potentially modify the activity of certain ATPases, e.g., myosin magnesium ATPase and F\textsubscript{1}-ATPase (10, 33, 68). PP\textsubscript{i} also facilitates iron transfer between transferrin and ferritin and iron delivery to mitochondria from transferrin (20). Certain previous overviews of PP\textsubscript{i} in mitochondrial metabolism merit attention (33, 68) for discussions of the ability of PP\textsubscript{i} to function in energy transfer reactions in yeast, where PP\textsubscript{i}, in combination with inorganic pyrophosphatase, generates a mitochondrial membrane potential (88). ATP-derived PP\textsubscript{i} also can function as a phosphate donor in protein phosphorylation in not only yeast mitochondria but also in mammalian cells (21). Such pyrophosphate-dependent kinase activity may be derived from an evolutionary precursor from which ATP-dependent protein phosphorylation also developed (90). Last, several less well-characterized biological effects of PP\textsubscript{i} in cell physiology appear to be exerted in the modulation of the fidelity and extent of DNA replication, protein synthesis, and calcium release from mitochondrial stores, as previously reviewed (68, 91). The significance of some of these effects in the cell physiology of higher organisms may be underestimated, even though effects of bisphosphonates on cell physiology are well recognized (28, 94, 101). Effects of dysregulated extracellular and intracellular PP\textsubscript{i} metabolism on cell physiology (in addition to effects of PP\textsubscript{i} on crystallization) may well contribute to the modulation by PP\textsubscript{i} of extracellular matrix calcification.

PP\textsubscript{i}, metabolism in matrix calcification

The main subjects of this review are the growing understanding of mechanisms by which skeletal cells make and dispose of PP\textsubscript{i}, and the direct regulatory role of the metabolism of PP\textsubscript{i} in skeletal matrix calcification. The work reviewed follows on the recognition, of over almost four decades, that PP\textsubscript{i} is a potent and direct inhibitor of crystallization in connective tissue matrices, the urinary tract, and in other extracellular fluids (2, 29–31, 41, 50, 52, 57, 83, 103, 106, 109, 125). For example, PP\textsubscript{i} directly inhibits the capacity of growth plate chondrocytes and osteoblasts to deposit minerals (specifically crystals of the basic calcium phosphate hydroxyapatite) in the pericellular matrix of bone (50–52, 89). Local PP\textsubscript{i} exclusion or degradation (74) appears to be necessary for mineralization to be seeded in osteoblast- and chondrocyte-derived membrane-limited structures termed matrix vesicles (MV\textsubscript{s}) (1, 2, 44, 45). Similarly, phosphocitrate inhibits the seeding of hydroxyapatite crystals in MV\textsubscript{s} (15, 16). The propagation of hydroxyapatite crystals outside MV\textsubscript{s}, which is mediated in part by the binding of P\textsubscript{i} to calcium associated with proteoglycans (89), also appears to be markedly suppressed by PP\textsubscript{i} (2). However, PP\textsubscript{i} does bind and promote stabilization of formed hydroxyapatite.

The ability of PP\textsubscript{i} to inhibit the formation of some calcium-containing crystals other than hydroxyapatite (including calcium oxalate) also appears physiologically significant. For example, urinary tract PP\textsubscript{i} serves as a natural inhibitor of urolithiasis (29), and measures to increase urine PP\textsubscript{i} see therapeutic use for oxalate urolithiasis. Furthermore, the ability of PP\textsubscript{i} to suppress calcification is utilized commercially via the supplementation with PP\textsubscript{i} (or with a variety of inorganic polyphosphates) of water softeners and of toothpaste preparations designed to inhibit the formation of dental calculus (30, 115). Inhibition of inorganic pyrophosphatase activity by fluoride may factor into calculus prevention by dentifrices containing fluoride as well as PP\textsubscript{i} or PP\textsubscript{i} analogs.
The effects exerted by PP\(_i\) on calcification in extracellular fluids and connective tissue matrices are influenced by a variety of factors, including the concentration of other solutes, tissue-specific and age-related changes in the overall protein and proteoglycan composition of the matrix, and other regulatory pathways including proteoglycan sulfation and matrix protein cross-linking and degradation (41, 58, 106). Importantly, PP\(_i\) supersaturation of the matrix in articular hyaline cartilage and meniscal fibrocartilage, and in certain tendons and ligaments, can directly promote matrix deposits composed of calcium pyrophosphate dihydrate (CPPD) crystals (106), as discussed further below. More detailed discussion of the mechanism of direct effects of PP\(_i\) on crystallization is beyond the scope of this review but can be accessed via references on the subject cited above.

In the last decade, it has become clear that numerous mediators that significantly impact on bone and cartilage mineralization may do so, at least in part, by modulating PP\(_i\) generation. These include the effects of calcitropic hormones, autacoid growth factors and cytokines, and altered cell signaling and differentiation (35, 36, 38, 42, 51, 65, 85–87, 96, 97, 104, 105, 112, 114, 119, 120).

**NTTPPPH ISOENZYMES IN PP\(_i\) GENERATION BY SKELETAL CELLS**

Molecular and functional characterization of NTTPPPH isoenzymes has mainly been done in chondrocytes to date. Chondrocytes in articular hyaline cartilages and meniscal fibrocartilages generate particularly high concentrations of extracellular PP\(_i\), and, similarly, have particularly high specific activities of NTTPPPH compared with cells from most other tissue types (51, 106). The constitutively high PP\(_i\) generation by the articular cartilages of diarthrodial joints and intraarticular menisci likely contribute to the fact that these cartilages do not physiologically calcify (106), unlike the case for growth plate cartilages.

Chondrocytes, as well as osteoblasts, express three PDNP family genes with NTTPPPH activity (35, 36): plasma cell membrane glycoprotein-1 (PC-1), autotaxin, and B10/PDNP3 (listed in Table 2 along with alternative designations). A chondrocyte-expressed protein that physically fractionated with NTTPPPH activity in a preliminary study has been named cartilage intermediate layer protein (CILP) (72). However, CILP has not been established to have intrinsic NTTPPPH activity. CILP is also unrelated to PDNPs and does not possess any of the structural features essential for NTTPPPH activity (62–64, 71).

The PC-1, autotaxin, and B10/PDNP3 genes each encode a class II (intracellular NH\(_2\) terminus) transmembrane glycoprotein ectozenzyme of 130 kDa with an extracellular domain containing two somatomedin B-like regions, a conserved calcium-binding EF hand, and a conserved PDNP catalytic site (12, 35, 36, 118). PC-1 is the most widely expressed of these NTTPPPH isoenzymes, but in vivo and in vitro observations indicate that many tissues express more than one NTTPPPH isoenzyme, but in vivo and in vitro observations indicate that many tissues express more than one NTTPPPH isoenzyme (35, 36, 51, 112, 116, 117). PC-1 appears to be needed to support ~50% of the normal levels of plasma PP\(_i\) and cultured fibroblast extracellular PP\(_i\) (103).

The cytosolic tail of PC-1, but not of autotaxin or B10/PDNP3, possesses a dileucine motif for targeting to the basolateral plasma membrane of polarized cells (18, 36, 55, 112). Correspondingly, PC-1 translocates to the basolateral surface and B10/PDNP3 to the apical surface in polarized cell types such as hepatocytes, and this differential localization is mediated by the cytosolic tail (112). Autotaxin is primarily secreted in a soluble form via proteolysis, but soluble forms of proteolytically derived PC-1 and B10 can be released into the extracellular fluid, and soluble, enzymatically active

Table 2. Human PP\(_i\)-generating PDNP family members and their major subcellular locations and functions in chondrocytes and osteoblasts

<table>
<thead>
<tr>
<th>PDNP Family Member</th>
<th>Other Designation(s) for Family Member</th>
<th>Sub-cellular Location(s)</th>
<th>Enzyme Activities</th>
<th>Established Functions in Skeletal Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC-1</td>
<td>E-NPP1, PDNP1, Npps, CD203a</td>
<td>Plasma membrane and MVs</td>
<td>NTTPPPH Nucleotide pyrophosphatase</td>
<td>Increases intracellular, extracellular, and MV PP(_i); antagonizes TNAP; physiological inhibitor of apatite deposition; PC-1 excess promineralizing in chondrocytes</td>
</tr>
<tr>
<td>Autotaxin</td>
<td>E-NPP2, PD-1a (alternative splice product), PDNP2, CD203b</td>
<td>Secreted</td>
<td>NTTPPPH T-type ATPase Nucleotide pyrophosphatase</td>
<td>Component of BMP-2-inducible gene expression program in skeletal development; promineralizing ? Housekeeping gene; elevates intracellular PP(_i), concentration</td>
</tr>
<tr>
<td>PDNP3</td>
<td>E-NPP3, B10, PD-1b, gp130RB13-6 CD203c</td>
<td>Intracellular</td>
<td>NTTPPPH Nucleotide pyrophosphatase</td>
<td></td>
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</table>

NTTPPPH, nucleoside triphosphate pyrophosphohydrolase (EC 3.6.1.8), catabolizes NTP → NMP + PP\(_i\). Nucleotide pyrophosphatase (EC 3.1.4.1) catabolizes sulfate transfer in proteoglycan synthesis via hydrolysis of the sulfate donor phosphoadenosine phosphosulfate (PAPS). Autotaxin unlike PC-1 or B10/PDNP3 catalyzes the phosphodiester bond on either side of the β-phosphate of ATP, acting as a threonine type (T-type) ATPase (EC 3.6.1.3). MV, matrix vesicle; TNAP, tissue-nonspecific alkaline phosphatase.
fragments of all three NTPPPH isoenzymes circulate in plasma (36, 103).

PC-1 (but not autotaxin or B10/PDNP3) preferentially localizes to the plasma membrane and to MVs in cultured chondrocytes and osteoblasts (50–52). The differential structures and localization of PC-1 and other NTPPPH isoenzymes have been determined to be associated with differential functions in matrix calcification. For example, elevated expression of PC-1, but not B10/PDNP3, elevates extracellular PPi in chondrocytes and, in osteoblasts, potently inhibits MV-mediated hydroxyapatite deposition (52). Circumstantial evidence points to a potential role of upregulated autotaxin expression in skeletal development at the stage of bone and growth cartilage ossification and tooth formation (4, 5). In addition, significant matrix calcification-promoting activity of transfected autotaxin has been detected in cultured chondrocytic cells (47). The capacity of autotaxin to promote matrix calcification likely reflects autotaxin induction of AP activity, the intrinsic P2-generating T-type ATPase activity of autotaxin (Table 2), and the weak capacity of autotaxin to increase extracellular PPi in chondrocytes (47).

CONSEQUENCES OF PC-1 DEFICIENCY FOR PPi, METABOLISM AND MATRIX CALCIFICATION

Recent work has elucidated that NTPPPH isoenzymes other than PC-1 are not sufficiently redundant with PC-1 on a functional level to replace the central role of PC-1 in the regulation of skeletal and arterial matrix calcification (78, 103, 108, 109). For example, in vitro studies have demonstrated that elevated B10/PDNP3 expression increases intracellular but not extracellular PPi in cultured chondrocytes and osteoblasts (51, 52). Yet, B10/PDNP3 does not appear to have major regulatory effects on matrix calcification in cultured chondrocytes or osteoblasts (47, 52). The study of skeletal PC-1 expression in mice (78, 108, 109) has been particularly revealing, because PC-1 has been demonstrated to exert physiologically critical inhibitory effects on skeletal mineralization at many of the sites where significant PC-1 expression is normally detected.

In mice, skeletal PC-1 expression occurs in osteoblasts, osteocytes, chondrocytes in articular hyaline and meniscal cartilages, and in articular and periaricular ligaments (109). Moreover, in growth plate cartilages, PC-1 is readily detected in epiphyseal regions in late hypertrophic chondrocytes in the calcifying zone. PC-1 is also strongly expressed at entheses (e.g., sites of insertion of intraarticular ligaments and the junction of synovial membrane with periosteum).

Both homozygous PC-1 null mice (109) and mice homozygous for a naturally occurring inactivating PC-1 truncation mutation (between the PC-1 catalytic site and EF hand domain) share an essentially identical phenotype termed “tiptoe walking” (ttw/ttw) (78, 108). The animals heterozygous for PC-1 in these models are phenotypically normal. In the PC-1-deficient mice, hyperossification occurs in early life and produces a progressive ankylosis of peripheral joints commencing at 3 wk of age that is responsible for the tiptoe gait (78, 108, 109). The PC-1-deficient mice also develop juvenile onset ankylosing ossification of periarticular ligaments, reminiscent of the human disorders ankylosing spondylitis, ankylosing hyperostosis, and ossification of the posterior longitudinal ligament (78, 108, 109). Calcification is particularly intense around intervertebral disks in the PC-1-deficient mice where the periosseum appears to mediate the hyperossification (108). The PC-1-deficient mice demonstrate extension of endochondral growth plates (109). The observation of dysregulated trabecular bone turnover in ttw/ttw animals prompted a therapeutic study of calcitonin administration that partially suppressed the development of cervical spine hyperostosis in 6-wk-old ttw/ttw mice (82).

The knee cruciate ligaments and the Achilles tendon also develop pathological calcifications in the PC-1-deficient mice (109). Furthermore, the PC-1-deficient mice develop diffuse deposition of hydroxyapatite in the articular and meniscal cartilages of joints with associated degenerative arthritis (108). In this light, particularly abundant calcified MV-like “chain granules” have been detected in cartilages of PC-1-deficient mice, reinforcing in vitro evidence (78, 108) for a central physiological role of MV-PC-1 in regulating the calcifying potential of MVs derived from skeletal cells (50).

The utility of PC-1-deficient mice as a model system for human disease has been underlined by the recent identification of a role for PC-1 deficiency in a human disorder of excess arterial matrix calcification. In this context, spontaneous arterial matrix calcification develops in early life in PC-1-deficient mice (78). Moreover, attenuated PC-1 gene expression, depression (by ~50%) of blood and cultured fibroblast NTPPPH levels, and intracellular and extracellular PPi levels were recently identified in a male human infant with idiopathic infantile arterial calcification (IIAC) (102, 103). This child presented clinically at 5 days of age with ischemic cardiomyopathy and renovascular hypertension. There was hydroxyapatite deposition with concomitant stenosing smooth muscle cell proliferation in large arteries by early infancy, classic features of IIAC (102, 103). In addition, there were dense periaricular calcifications of wrists and ankles, a feature of a subset of up to 20% of IIAC patients (102, 103). This subject responded therapeutically to bisphosphonates, as reported for a significant number of subjects with IIAC (103).

PC-1 has phosphoadenosine sulfate-hydrolyzing nucleotide pyrophosphatase activity that can affect protein N-glycosylation and matrix proteoglycan sulfation (36). PC-1, like other NTPPPH isozymes of the PDNP family, can also hydrolyze nucleoside diphosphates, dinucleoside polyphosphates, and nucleoside monophosphate esters, and PC-1 is believed to play a significant role in modulating signaling by extracellular ATP (35, 36, 39). PC-1 also has been reported to exert nonenzymatic effects on insulin signaling (37).
have elevated intracellular PP\textsubscript{i} and low extracellular PP\textsubscript{i} levels in regulation of matrix calcification. In addition, there appears to be a mutually antagonistic regulatory loop involving PP\textsubscript{i} and P\textsubscript{i}. Specifically, P\textsubscript{i} generation by tissue-nonspecific alkaline phosphatase (TNAP) through PP\textsubscript{i} hydrolysis appears to be a central mechanism (52) by which TNAP exerts physiologically essential stimulatory effects on mineralization of the bone matrix (79, 123, 124, 128). Conversely, PC-1 directly antagonizes the stimulatory effect of TNAP on osteoblast MV-mediated calcification in an MV PP\textsubscript{i}-dependent manner (52).

**ANK IN PP\textsubscript{i} METABOLISM AND MATRIX CALCIFICATION**

A significant fraction of extracellular PP\textsubscript{i} appears to be derived by channeling from the cell interior by a mechanism that involves the widely expressed 54-kDa protein ANK (43). The multiple-pass transmembrane structure of ANK, and functional findings in ANK-deficient and ANK-sufficient cells, have suggested that ANK could be a component of probenecid-suppressible anion transport channel for PP\textsubscript{i} (43). Whether ANK might also modulate movement to the cell exterior of anion transport channel for PP\textsubscript{i} (43). Whether ANK might also modulate movement to the cell exterior of the NTPPPH substrate ATP is not known.

Truncation mutation of the NH\textsubscript{2}-terminal cytosolic domain of ANK in ank/ank mice causes a hypermineralizing phenotype (murine progressive ankylosis) remarkably similar to that of PC-1-deficient mice (43, 57). However, cultured fibroblasts of ank/ank mice have elevated intracellular PP\textsubscript{i} and low extracellular PP\textsubscript{i} levels, which are both corrected by transfection of wild-type ANK (43). In contrast, intracellular and extracellular PP\textsubscript{i} levels are both low in PC-1-deficient cultured fibroblasts (103). These particular observations, in addition to the salutary therapeutic effects for murine progressive ankylosis of administration of the PP\textsubscript{i} analog phosphocitrate (57), have particularly strengthened the case for extracellular PP\textsubscript{i} being a major determinant of mineralization.

**DYSREGULATED PP\textsubscript{i} METABOLISM IN MATRIX CALCIFICATION DISORDERS: FURTHER CONSIDERATIONS**

This review has discussed several hypercalcifying disorders in which depression of extracellular PP\textsubscript{i} levels appear to pay a central role (Table 3). It is conceivable that diffuse spinal ligamentous calcification in inflammatory spondyloarthropathies such as ankyllosing spondylitis could also be mediated in part by inflammation-induced suppression of PC-1 expression and PP\textsubscript{i} generation by ligament fibroblasts encasing the spine. In this regard, the inflammatory mediators interleukin (IL)-1 and tumor necrosis factor (TNF)-\alpha suppress PC-1 expression and induce decreases in extracellular PP\textsubscript{i} in chondrocytes, osteoblasts, and potentially other cells (65, 108).

PC-1 expression is growth regulated, controlled by an mRNA-stabilizing protein that has a high turnover (117), and is subject to transcriptional regulation by TGF-\beta, basic fibroblast growth factor, IL-1, and other mediators (36, 51, 65, 114). Certain mediators of mineralization, including parathyroid hormone-related protein (PTHRP) 1–173 and insulin-like growth factor I (IGF-I), suppress extracellular PP\textsubscript{i} levels (without affecting NTPPPH activity) in chondrocytes (38, 85, 119). Thus we also speculate that modulation of PC-1 and/or ANK expression and extracellular PP\textsubscript{i} levels by not only IL-1 and TNF-\alpha, but also by IGF-I and PTHRP 1–173 (or other regulators of cell differentiation including bone morphogenetic proteins) (120), play a role in hydroxyapatite deposition in osteoarthritic cartilage (41) and in certain disorders with widespread hyperossification of unknown etiology. This line of study may

Table 3. Diseases of matrix calcification currently attributed to disregulated PP\textsubscript{i} metabolism

<table>
<thead>
<tr>
<th>Disease State</th>
<th>Molecular Abnormality</th>
<th>Alterations in PP\textsubscript{i} Metabolism</th>
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<tr>
<td>Murine progressive ankylosis</td>
<td>Homozygosity for ANK truncation mutation</td>
<td>Increased intracellular and decreased extracellular PP\textsubscript{i}</td>
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<tr>
<td>Tiptoe walking mice</td>
<td>Homozygosity for PC-1 truncation mutation and for PC-1 gene knockout</td>
<td>Decreased intracellular and extracellular PP\textsubscript{i}</td>
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<tr>
<td>Human arterial calcification of infancy with periarticular calcification</td>
<td>Deficient PC-1 expression (mutation not yet identified)</td>
<td>Decreased intracellular and extracellular PP\textsubscript{i}</td>
</tr>
<tr>
<td>Hypophosphatasia</td>
<td>TNAP mutations</td>
<td>Increased extracellular PP\textsubscript{i}</td>
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<tr>
<td>Chondrocalcinosis (CPPD deposition disease) secondary to hemochromatosis, hyperparathyroism, hypomagnesemia (as well as hypophosphatasia)</td>
<td>Likely heterogeneous</td>
<td>Increased extracellular PP\textsubscript{i} in joints</td>
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<tr>
<td>Idiopathic/sporadic aging-associated chondrocalcinosis (CPPD crystal deposition disease)</td>
<td>Linkage to chromosome 5p in some cases (? ANK dysfunction)</td>
<td>Increased cartilage intracellular and extracellular PP\textsubscript{i}, and NTPPPH activity, increased extracellular ATP (an NTPPPH substrate) in joints</td>
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<tr>
<td>Familial chondrocalcinosis</td>
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<td>Variable (increased intracellular PP\textsubscript{i} described in some kindreds)</td>
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CPPD, calcium pyrophosphate dihydrate.
be compelling for not only ankylosing spondylitis but also two other spinal hyperostotic disorders: ossification of the posterior longitudinal ligament and diffuse idiopathic skeletal hyperostosis (78).

As would be anticipated from the pathological consequences of extracellular PPi deficiency, an excess of extracellular PPi, in hypophosphatasia (TNAP deficiency) (79, 123), is associated with impaired bone matrix hydroxypatite deposition (ostemalacia) (Table 3). However, the matrix of joint cartilages, unlike the matrix of bone, can support calcification with crystals composed of calcium and PPi (CPPD) (106). Moreover, paradoxical effects of PPi on matrix calcification are illustrated by the observation that subjects with hypophosphatasia can develop pathological mineralization of articular cartilage (with CPPD) despite manifesting undermineralization of bone (53). In hypophosphatasia as well as hemochromatosis, hyperparathyroidism, and hypomagnesemia (Table 3), articular cartilage CPPD deposition is promoted by mechanisms that include intraarticular elevation of extracellular PPi, resulting, in part, from impairment of inorganic pyrophosphatase activity (53, 106).

Chondrocalcinosis most commonly arises as a primary, idiopathic (and particularly prevalent) condition associated with aging (106). In this context, articular cartilage NTPPPH activity and PPi elaboration increase markedly with cartilage aging (91, 95, 98), and these changes are strongly linked to idiopathic/sporadic chondrocalcinosis (106). Significantly, TGF-β potently induces increased extracellular PPi in articular chondrocytes, an activity that appears to be mediated in large part by both TGF-β-induced PC-1 expression and PC-1 translocation to the plasma membrane (51, 65). Moreover, TGF-β-induced increases in chondrocyte extracellular PPi are augmented in an age-dependent manner in association with a decrease in proliferative responses of aging chondrocytes to TGF-β (95), suggesting that altered TGF-β signaling, along with increased NTPPPH activity in aging chondrocytes, impact directly on PPi metabolism. Other mechanisms may also contribute to elevated PPi generation by aging cartilage, including increased extracellular availability of the NTPPPH substrate ATP (107) and altered mitochondrial PPi metabolism in aging (3), as illustrated by the observation that PPi concentrations also increase in blood platelets in association with human aging (106).

Heterogeneous familial forms of early onset chondrocalcinosis have been described (46, 53, 93, 106). In some of the described families, the term chondrocalcinosis has been loosely applied to describe excess calcium-containing crystal deposits in cartilage and soft tissues that actually represent hydroxypatite and/or CPPD. Some of these familial phenotypes likely involve production of an altered cartilage matrix (93, 106). However, dysregulated PPi metabolism has been established in several of these kindreds (93, 106). It is suspected that abnormalities in PC-1, other NTPPPH activities, or ANK function could be at play in certain families with premature chondrocalcinosis. For example, chromosomal linkage to 5p (the region where the ANK gene is located) has been established in two families with chondrocalcinosis (46, 69, 93). Interestingly, childhood epilepsy is a feature of familial chondrocalcinosis linked to chromosome 5p (46), and toxic effects of excess intracellular PPi on central nervous system cells could be responsible, although deficient ANK function has not yet been identified in this kindred.

Elevated expression of PC-1 is topographically associated with both chondrocyte apoptosis and MV-mediated matrix calcification in situ in knee meniscal cartilages with idiopathic CPPD crystal deposition disease (47). A similar elevation of PC-1 expression paradoxically stimulates hydroxypatite crystal deposition by cultured chondrocytes in vitro (47). Moreover, cartilage hydroxypatite deposits commonly occur in conjunction with CPPD crystal deposits in chondrocalcinosis, despite the local abundance of the hydroxypatite deposition inhibitor PPi (41, 106).

The capacity of elevated PC-1 activity to paradoxically augment matrix calcification by chondrocytes likely reflects chondrocyte-specific factors beyond the production of matrix PPi supersaturation (as schematized in Fig. 2). The provision by PPi of a source of Pi, through pyrophosphatase activities, is likely to be involved, because PPi is a modulator of cell differentiation and gene expression (9, 19, 73). In addition, excess PPi (likely through effects mediated by the plasma membrane sodium phosphate exchanger and mitochondrial calcium uptake and function) can induce apoptosis (67, 73), which is promineralizing in chondrocytes (48, 56).

PPi regulates crystallization, but effects of PPi on matrix calcification may not be simply limited to activities exerted at the level of mineral crystal seeding in MVs or propagation of crystals in the matrix. Indeed, the extent to which intracellular and extracellular PPi concentrations modulate cell physiological functions in higher organisms may be underestimated at present. In contrast, several specialized mechanisms for PPi generation, transport, and degradation have been as-

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**Deficient PC-1**  
**Physiologic PC-1**  
**Elevated PC-1**  

![Fig. 2](http://apcell.physiology.org.by/10.222.033.1/28/2017)

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**Fig. 2.** Model for occurrence of pathological articular cartilage matrix calcification in states of both articular chondrocyte plasma cell membrane glycoprotein-1 (PC-1) expression deficiency and excess. Model schematizes the putative basis for the findings that pathological articular cartilage matrix calcification occurs in states of both PC-1 deficiency ("tiptoe walking mice (ttw/ttw") and PC-1 excess (idiopathic chondrocalcinosis of aging), as discussed in detail in the text. Pi, inorganic phosphate; CPPD, calcium pyrophosphate dihydrate.
certained to have important roles in the regulation of physiological and pathological skeletal calcification and pathological arterial matrix calcification. Moreover, certain molecular and biochemical abnormalities of PPi metabolism have been identified as pathogenic factors in certain hereditary and aging-associated diseases. Recently characterized mediators of PPi metabolism include PC-1 and ANK. PC-1 has been defined to have an antagonistic functional relationship with TNAP at the level of MV PPi in matrix calcification. Intriguingly, TNAP regulates the expression of PC-1, which suggests the possibility that PPi (in addition to P) (9, 19, 73) could be a component of sensing mechanisms that modulate gene expression and cell differentiation. Further evaluation of the regulatory relationships between TNAP, PC-1, ANK, and PPi in cell physiology, skeletal development, and matrix calcification should prove interesting.

Pathologically similar associations of deficient ANK and PC-1 function with excessive matrix calcification and hyperostosis in mice have been characterized. Moreover, a PC-1-deficient human has been observed to have a hypercalcifying phenotype similar to that of PC-1-deficient mice. Paradoxically, excess PC-1 expression also has been linked to the pathogenesis of chondrocalcinosis. One anticipates that systemic and localized “gain of function” and “loss of function” of ANK and PC-1 will be implicated in an expanding number of clinical disorders in humans.

REFERENCES


side triphosphate pyrophosphohydrolase (NTPP-PH) isozyme PC-1 in idiopathic infantile arterial calcification. Am J Pathol.


