Regulation of bone resorption and formation by purines and pyrimidines

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Growing evidence suggests that extracellular nucleotides, signalling through P2 receptors, might play important roles in the regulation of bone and cartilage metabolism. ATP and other nucleotides can exert impressive stimulatory effects on the formation and activity of osteoclasts (bone-resorbing cells) in addition to inhibiting bone formation by osteoblasts. In this review, the current understanding of the actions of nucleotides on skeletal cells and the probable receptor subtypes involved are discussed.

Bone is a highly specialized form of connective tissue that, together with cartilage, makes up the skeletal system. It is composed of inorganic mineral salts deposited within an organic collagen matrix, and three major cell types: osteoclasts, osteoblasts and osteocytes (Table 1; Fig. 1). Bone is a dynamic, living tissue; continuous modelling and remodelling by bone cells allows the skeleton to grow and adapt. Abnormalities of bone remodelling can produce a variety of skeletal disorders.

Osteoblasts are mononuclear cells of mesenchymal origin that are responsible for bone formation. They are able to secrete an extracellular matrix consisting mainly of type I collagen, which they later mineralize. The periosteum and bone marrow are important sources of mesenchymal osteoprogenitor cells. Osteoblasts that are actively secreting bone matrix are large cuboidal mononuclear cells with a prominent protein synthesizing apparatus, whereas the quiescent osteoblasts that cover most adult bone surfaces have a flat morphology. Some osteoblasts become incorporated in the bone matrix they secrete, differentiating into osteocytes, which form a regular, interconnected network of cells that is thought to mediate responses to mechanical loading. In contrast to cartilage, bone is highly vascular; the blood vessels and nerve fibres that ramify through bone constitute an important, albeit poorly understood, regulatory system.

Osteoclasts have the unique ability to resorb bone extracellularly, a process that entails excavation of characteristic pits and troughs on bone surfaces. Osteoclasts are multinucleated cells formed by the proliferation of haematopoietic, mononuclear progenitors of the monocyte and macrophage lineage and their subsequent fusion into multinucleated osteoclasts. Two molecules, produced by stromal cells, have now been identified and shown to be both essential and sufficient for osteoclastogenesis: macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor κB ligand (RANKL). The sequence of events required for bone resorption involves migration of osteoclasts to the site of resorption, followed

Table 1. Characteristics of bone cells

	Osteoblasts (bone-forming cells)	Osteoclasts (bone-resorbing cells)
Origin	Derived from precursors in periosteum and bone marrow stroma; common stromal precursor also gives rise to fibroblasts, adipocytes and chondrocytes	Derived from pro-monocytic precursors in bone marrow, spleen and peripheral blood; generally multinucleated
Function	Secrete and mineralize bone organic matrix (~90% type I collagen)	Unique ability for extracellular resorption of mineralized tissues; rapidly excavate characteristic pits and troughs on bone surfaces
Markers	Alkaline phosphatase, osteocalcin and osteonectin	Tartrate resistant acid phosphatase, carbonic anhydrase II, vitronectin receptor and calcitonin receptor
Differentiation and proliferation	Differentiate into osteocytes (network of strain-detecting cells) when engulfed by bone matrix; primary cells (e.g. from rodents or humans) proliferate readily for a few weeks in culture; form mineralized collagenous bony nodules at high density in long-term cultures (~3 weeks)	Primary cultures containing precursors (e.g. from mouse bone marrow or human peripheral blood) can differentiate into multinucleated functional osteoclasts *in vitro* (~2 weeks)
Cell lines	Many transformed 'osteoblast-like' cell lines are available, mostly derived from osteosarcomas (e.g. ROS 17/2.8, MG63 and UMR106); cell lines have limited osteogenic potential *in vitro*	Some transformed macrophage-like cell lines (e.g. RAW 264.7) differentiate into 'osteoclast-like' cells but appear to be unable to resorb bone *in vitro*

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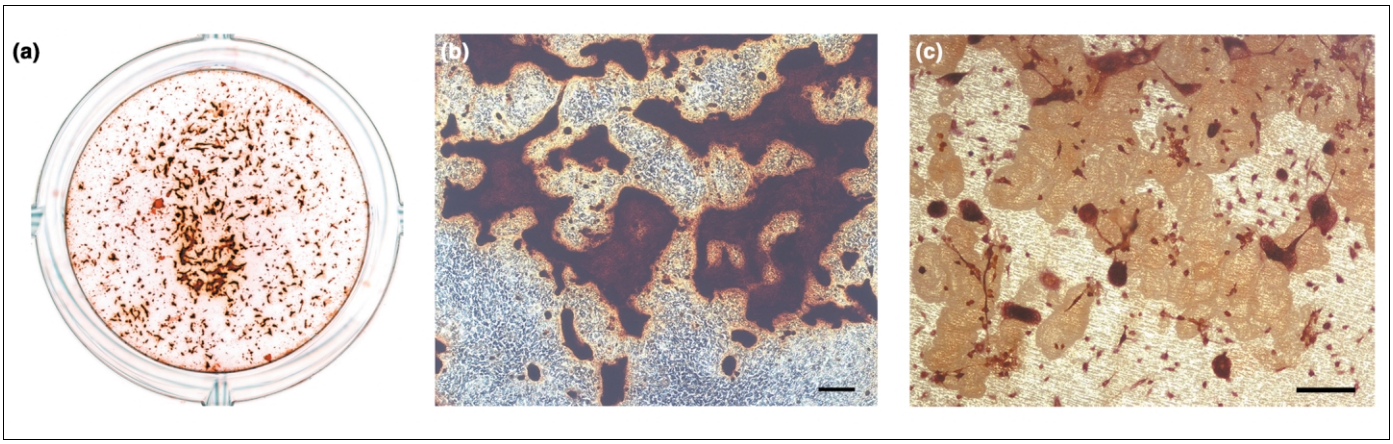

Fig. 1. Bone cell culture systems. (a,b) Bone nodules in a 21-day culture of rat calvarial osteoblasts (bone-forming cells) are shown. (a) A low-power image of a culture well (from a 24-well plate) after staining with alizarin red demonstrates mineralization by the osteoblasts. (b) A higher power image of (a), using phase-contrast microscopy, illustrates the characteristic nodule morphology; nodules (dark red-brown) are surrounded by a monolayer of confluent osteoblasts (blue-grey). Scale bar = 200 μM. (c) Mouse bone marrow cultures on ivory (dentine) substrate are shown. Cells were stained to demonstrate tartrate resistant acid phosphatase (TRAP) after 10 days of culture, and viewed by reflected light microscopy. Numerous multinucleated, TRAP-positive osteoclasts (bone-resorbing cells; large, dark red cells) have formed in the culture and then excavated large, contiguous resorption pits in the bone (tan areas with scalloped boundaries). Scale bar = 100 μM.

by their attachment to the bone, polarization and the formation of a sealed extracellular vacuole into which protons (H^+ ions) and enzymes (chiefly cathepsin K) are secreted by the osteoclasts to dissolve bone mineral and degrade the collagenous organic matrix.

The idea that purines could act as extracellular signalling molecules was first proposed >80 years ago [1]. Extracellular nucleotides have since been implicated in a wide range of biological processes, including smooth muscle contraction, inflammation, platelet aggregation and pain, among many others [2]. Receptors for purines and pyrimidines have been classified into two groups (Box 1; Table 2). In this review, current understanding of the actions of nucleotides on skeletal cells, the probable receptor subtypes involved and possible pathophysiological implications are summarized.

Box 1. Purine and pyrimidine receptors

Classification

Receptors for purines and pyrimidines have been classified into two groups: P1 receptors with adenosine as the main ligand, and P2 receptors with ATP, ADP, UTP and UDP as the main ligands. On the basis of pharmacology, cloning and transduction studies, ionotropic ligand-gated ion channels $P2X_{1-7}$ and metabotropic G-protein-coupled $P2Y_{1,2,4,6,11,12,13,14}$ receptor families were established (see Table 2 in the main text). P2X receptor subunits consist of two hydrophobic transmembrane domains, a large *N*-glycosylated extracellular loop and intracellular N- and C-termini. At least three (or four) subunits are thought to form a functional P2X receptor channel. P2Y receptors consist of seven transmembrane domains, with an extracellular N-terminus and an intracellular C-terminus. (For more detailed recent reviews on the pharmacology and distribution of these receptors, see [2,58]).

Sources and fate of nucleotides

Nucleotides are present intracellularly at a concentration of ~2–5 mM. Several cells, including tumour cells, platelets, endothelial and epithelial cells, release ATP upon mechanical stimulation. Intracellular ATP can be released [59,60] by: (1) cytolysis after cell damage or cell death following physical or biological trauma; (2) vesicular release from nerve terminals and from some non-neuronal cells; (3) ATP-binding cassette (ABC) proteins; (4) connexin hemichannels, as reported for astrocytes [60]. Once released, the action of nucleotides at their receptors is terminated by a cell-surface-located enzyme cascade (ecto-nucleotidases) that sequentially degrades nucleoside 5′-triphosphates to their respective nucleoside 5′-di and -monophosphates, nucleosides and free phosphates or pyrophosphate, which can all appear in the extracellular fluid at the same time.

Role of P2 receptors in osteoclast biology

The first evidence that osteoclasts respond to nucleotides came from studies using cultured rabbit osteoclasts. Adenosine 5′-triphosphate (ATP) was shown to elicit an increase in the concentration of intracellular Ca^{2+} $[Ca^{2+}]_i$ in these cells via influx of Ca^{2+} across the cell membrane and G-protein-coupled release of Ca^{2+} from internal stores [3,4]. Subsequent electrophysiological studies provided evidence for the coexistence of both P2X and P2Y receptors on osteoclasts [5]. More recent studies reported that the

Table 2. Characteristics of P2 receptors

Receptor	Agonists[a]	Special characteristics
P2X		
$P2X_1$	αβmeATP = ATP = 2meSATP	Rapid desensitization
$P2X_2$	ATP ≥ ATPγS ≥ 2meSATP ≫ αβmeATP	Sensitive to pH and Zn^{2+}
$P2X_3$	2meSATP ≥ ATP ≥ αβmeATP	Rapid desensitization
$P2X_4$	ATP ≫ αβmeATP	–
$P2X_5$	ATP ≫ αβmeATP	–
$P2X_6$	–	Does not function as a homomultimer
$P2X_7$	BzATP > ATP ≥ 2meSATP ≫ αβmeATP	Large pore with prolonged activation
P2Y		
$P2Y_1$	2meSADP > 2meSATP = ADP > ATP	–
$P2Y_2$	UTP = ATP	–
$P2Y_4$	UTP ≥ ATP	–
$P2Y_6$	UDP > UTP ≫ ATP	–
$P2Y_{11}$	ARC67085MX > BzATP ≥ ATPγS > ATP	–
$P2Y_{12}$	ADP	–
$P2Y_{13}$	2meSADP ≥ ADP	–
$P2Y_{14}$	UDP-glucose	–

[a]Abbreviations: ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; ATPγS, adenosine 5′-3-O-thiotriphosphate; BzATP, 2′,3′-*O*-(4-benzoyl-benzoyl) ATP; αβmeATP, α,β-methylene ATP; 2meSADP, 2-methylthioADP; 2meSATP, 2-methylthioATP; UDP, uridine 5′-diphosphate; UTP, uridine 5′-triphosphate.

adenosine 5′-diphosphate (ADP) analogue ADPβS elicited a Ca^{2+}-dependent K^+ current in rabbit osteoclasts, and raised $[Ca^{2+}]_i$ in rat osteoclasts, consistent with the presence of the $P2Y_1$ receptor on osteoclasts [6,7].

Using immunocytochemistry and *in situ* hybridization techniques on rat bone sections and cultured rat bone cells, evidence for the expression of $P2X_2$, $P2X_4$, $P2Y_1$ and $P2Y_2$ receptor subtypes on osteoclasts was found [8]; detection of the $P2X_4$ receptor was consistent with electrophysiological and reverse-transcriptase polymerase chain reaction (RT–PCR) evidence [6]. In addition, cultured rat osteoclasts show nuclear staining for the $P2X_7$ receptor [8,9] (the possible functional significance of this finding will be discussed below). A recent study using RT–PCR found evidence for the expression of a wider range of P2 receptors ($P2X_{1,4,5,6,7}$ and $P2Y_{1,2,4,6,11}$ receptors) on normal human osteoclasts, but no evidence for $P2X_2$ receptor expression [10].

A potential role for P2 receptors in osteoclast biology was first proposed in 1995 when ATP was shown to stimulate bone resorption by cells derived from a human osteoclastoma or 'giant cell tumour' [11]. Although it was proposed that this action might be mediated by the $P2Y_2$ receptor [11], this could not be confirmed in a follow-up study because the potent $P2Y_2$ receptor agonist uridine 5′-triphosphate (UTP), in contrast to ATP, failed to stimulate bone resorption [12]. Subsequently, ATP, at low concentrations, was shown to stimulate not only the resorptive activity of osteoclasts but also the formation of rodent osteoclasts. The stimulatory effect of ATP on resorption was amplified greatly when rat osteoclasts were co-activated by culture in acidified medium [13]. This suggested the possible involvement of the acid-sensitive $P2X_2$ receptor, the only P2 receptor that elicits a significant increase in ATP-evoked currents when the pH is lowered to below 7.0 [14].

Recently, the first evidence to link a specific P2 receptor to the action of nucleotides on bone resorption was reported [15]. Extracellular ADP and 2-methylthioADP (2-meSADP), a selective $P2Y_1$ receptor agonist, were shown to be potent stimulators of bone resorption at nanomolar to low micromolar concentrations, as assessed by three independent methods [15]. The actions of ADP on resorption pit formation by mature rat osteoclasts were biphasic: no effects were evident at higher concentrations (20–200 μM), which is in agreement with a bell-shaped response curve observed earlier for ADP at the $P2Y_1$ receptor [16]. Adenosine 5′-monophosphate (AMP) and adenosine had no significant effect on resorption, which suggests that ADP itself was the signalling agent. The ADP effect could be blocked in a non-toxic manner by the compound MRS2179 (see Chemical names), one of the most potent $P2Y_1$ receptor antagonists reported to date [17]. The experiments indicated that extracellular ADP could stimulate resorption directly via the $P2Y_1$ receptor expressed on mature osteoclasts or indirectly via receptors expressed on osteoblasts, which in turn release pro-resorptive local factors, or by both direct and indirect mechanisms. A recent study on human osteoclasts suggested that the effect of ATP on resorption is indirect through upregulation of RANKL in osteoblasts [10]. Experiments using mouse marrow cultures also indicated that ADP stimulates osteoclast formation from haematopoietic precursors, in addition to activating mature osteoclasts. In mouse calvarial bone organ cultures, resorption stimulated by ADP was blocked by the cyclooxygenase inhibitor indomethacin, suggesting a requirement for endogenous prostaglandin synthesis in this culture system. A similar dependency on prostaglandins has been observed for other osteolytic agents, such as protons, in calvarial organ cultures [18]. Two earlier studies also investigated the actions of ADP on osteoclasts but at much higher concentrations: ADP at 50 μM increased $[Ca^{2+}]_i$ and ADP at 100 μM induced a decrease in the intracellular pH in rabbit osteoclasts [4,19], whereas interestingly ADP appears to exert its major pro-resorptive action on osteoclasts at much lower concentrations of between 20 nM and 2 μM.

As mentioned above, ATP is also a potent stimulator of the activation and formation of rodent osteoclasts, an effect only evident at low pH (~6.9), which suggests the involvement of the $P2X_2$ receptor [13]. However, a similarly low pH is also required for the stimulatory effect of ADP, acting through the non-acid-sensitive $P2Y_1$ receptor [15]. This is consistent with earlier studies showing that the pro-resorptive effects of other agents are acid dependent [20], and thus suggests that there is a universal dependency of osteolytic agents on slight local acidification for their action. Whether the $P2X_2$ receptor plays a general role in mediating this process remains to be determined.

Osteoclasts have been reported to undergo cell death when exposed to high concentrations of ATP (1–2 mM) [13]. Apart from initiating active cell death, activation of $P2X_7$ receptors could also be involved in a different process in osteoclast biology: the receptor has been implicated in the formation of giant cells by mediating the fusion of murine macrophage-like cells [21]. It is therefore conceivable that the fusion of osteoclast precursors is also initiated by $P2X_7$-receptor-mediated pore formation in the membranes of adjacent cells, leading to the development of cytoplasmic bridges. However, high concentrations of ATP can also cause the development of a slowly inactivating inward current that is permeable only to small cations; this rules out pore formation and suggests yet another unknown role for the $P2X_7$ receptor in osteoclast biology [9]. Table 3 summarizes the evidence and possible functions of P2 receptors in osteoclasts.

Role of P2 receptors in osteoblast biology

Several studies have shown that nucleotides act through P2 receptors to induce formation of inositol (1,4,5)-trisphosphate [Ins(1,4,5)P_3] and transiently elevate $[Ca^{2+}]_i$ in osteoblastic cells [22–24]. Studies on rat osteoblast-like cells demonstrated that extracellular nucleotides interact with at least two receptor subtypes; the pharmacological profiles were characteristic of $P2Y_1$- and $P2Y_2$-like receptors [25–27]. Studies on single cells and populations

Chemical names

MRS2179: 2′-deoxy-N^6-methyladenosine-3′,5′-bisphosphate

Table 3. Evidence and possible functions for P2 receptors in osteoblasts and osteoclasts[a]

Receptor subtype	Species	Evidence	Refs[b]	Proposed function[c]
Osteoblasts				
$P2X_2$	Rat	Immunolabelling and *in situ* hybridization	[8]	–
$P2X_5$	Rat	Immunolabelling	[8]	Proliferation, differentiation
	Human	RT–PCR	[30]	
$P2X_6$	Human	RT–PCR	[30]	–
$P2X_7$	Human	RT–PCR	[30]	Active cell death at high ATP concentrations
	Human	Immunolabelling and RT–PCR	[32]	
P2Y	Rat	Ca^{2+} release from stores	[25–27]	
$P2Y_1$	Rat	*In situ* hybridization	[8]	Enhance PTH-induced Ca^{2+} signalling; release of pro-resorptive factors (e.g. prostaglandins and RANKL)
	Human	RT–PCR	[29]	
$P2Y_2$	Rat	*In situ* hybridization	[8]	Inhibition of bone formation; intercellular communication between osteoblasts
	Human	RT–PCR	[11,29]	
$P2Y_4$	Human	RT–PCR	[29]	–
$P2Y_6$	Human	RT–PCR	[29]	–
Osteoclasts				
P2X	Rat	Ca^{2+} influx	[5]	
$P2X_2$	Rat	Immunolabelling and *in situ* hybridization	[8]	Increased osteoclast activity
$P2X_4$	Rat	Immunolabelling and *in situ* hybridization	[8]	–
	Rat	Nonselective cation current	[5]	
	Rabbit	RT–PCR and cation current	[6]	
$P2X_7$	Rat	Immunolabelling	[8]	Intercellular communication between osteoblasts and osteoclasts; fusion of osteoclast progenitors; active cell death (at high ATP concentrations)
	Mouse	Permeabilization	[33]	
	Rabbit	Nonselective cation current	[9]	
	Human	RT–PCR	[56]	
P2Y	Rabbit	Ca^{2+} release from stores	[3,7]	
	Rat	Ca^{2+} release from stores	[5,7,57]	
$P2Y_1$	Rat	*In situ* hybridization	[8]	Increased osteoclast formation; increased resorptive activity
$P2Y_2$	Rat	*In situ* hybridization	[8]	–
	Human	RT–PCR	[11]	
	Human	*In situ* hybridization	[12]	

[a]Abbreviations: ATP, adenosine 5′-triphosphate; PTH, parathyroid hormone; RANKL, receptor activator of nuclear factor κB ligand; RT–PCR, reverse-transcriptase polymerase chain reaction.
[b]The references cited relate to the evidence for the presence of the receptor subtypes in osteoblasts and osteoclasts.
[c]The proposed functions relate to the specific receptor subtypes.

of human osteoblasts revealed heterogeneity of receptor expression within one cell culture [28]. This might indicate that expression of P2 receptors changes during the osteoblast life cycle, depending on the differentiation state.

The first molecular evidence for the expression of P2Y receptors by osteoblasts came with the localization by *in situ* hybridization of $P2Y_2$ receptors [11] in human osteoblasts and RT–PCR evidence for $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors in human osteosarcoma cell lines [29]. More recently, evidence for the expression of $P2X_2$, $P2X_5$, $P2Y_1$ and $P2Y_2$ receptors, at protein and mRNA levels, on rat osteoblasts was reported [8]. P2X receptor expression has also been described in human osteoblasts, and the $P2X_5$ receptor has been implicated in the stimulation of DNA synthesis by ATP [30]. Earlier studies showed that $P2X_5$ receptor immunoreactivity was indeed restricted to the metabolically active, differentiating cell layers in epithelia and hair follicles [31]. Thus, $P2X_5$ receptors might participate in the regulation of osteoblastic proliferation and differentiation.

There are conflicting data on the expression of the $P2X_7$ receptor in osteoblasts. The presence of $P2X_7$ receptors in primary human osteoblasts has been described [32], whereas no evidence for $P2X_7$ receptor expression was found on rat osteoblasts [8]. An earlier study reported that high ATP concentrations caused formation of pores in murine osteoclasts and macrophages, but not in osteogenic or chondrogenic cells [33]. Thus, the potential role and presence of the $P2X_7$ receptor in osteoblasts remains to be clarified.

Both ATP and adenosine are able to act as mitogens for osteoblastic cells; their mitogenic effects might be mediated indirectly through the enhancement of prostaglandin E (PGE) synthesis by ATP. Additionally, several studies have reported that nucleotides could act synergistically with growth factors such as platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF) to induce osteoblast proliferation [34].

Activation of $P2Y_1$ and $P2Y_2$ receptors has been shown to potentiate subsequent parathyroid hormone (PTH) receptor-mediated Ca^{2+} signalling [35,36]. For example, it has been suggested that PTH receptors are capable of activating adenylyl cyclase but might be unable to activate phospholipase C until cells receive a signal as a consequence of P2 receptor activation [37]. These synergies suggest a mechanism through which systemic PTH could initiate bone remodelling at specific sites in the skeleton by cooperating with the localized release of nucleotides. Thus, one could speculate that in damaged bone tissues increased local levels of PDGF and nucleotides released from activated platelets, endothelial cells and other cells, attract osteogenic cells to lesional sites and stimulate their proliferation. Extracellular nucleotides present in the bone microenvironment might thus be capable of modulating bone cells and controlling the remodelling process by interacting with,

and potentiating, both systemic hormones, such as PTH, and local growth factors [37].

Furthermore, extracellular nucleotides have been shown to reduce the amount of bone formed by primary rat osteoblasts in a novel *in vitro* appositional bone formation model [38]. This study used relatively high concentrations of ATP (50–500 μM) and results for the effects of UTP were equivocal, so that it was not possible to infer which receptor subtypes might be involved. More recently, the actions of nucleotides on bone formation by osteoblasts were re-examined using a different, more conventional model. Both UTP and ATP, at concentrations as low as 1–10 μM, but not adenosine or ADP, caused strong inhibition of mineralized bone nodule formation by cultured rat osteoblasts [39]. The potent inhibitory actions of ATP and UTP point to the involvement of either $P2Y_2$ or $P2Y_4$ receptors. No evidence for the expression of the $P2Y_4$ receptor subtype on rat osteoblasts was found, which suggests that the $P2Y_2$ receptor might mediate these inhibitory effects [8]. The earlier observation by Jones *et al.* that adenosine 5′-O-3-thiotriphosphate (ATPγS), a potent agonist at the $P2Y_2$ receptor, also inhibited osteoblastic bone formation is consistent with the notion that the effect is mediated via the $P2Y_2$ receptor [38].

$P2Y_2$ receptors have recently been shown to mediate oscillatory fluid flow-induced Ca^{2+} mobilization in murine osteoblasts [40]. Mechanically stimulated human osteoblasts have also been shown to propagate fast intercellular Ca^{2+} waves via autocrine activation of $P2Y_2$ receptors [41]. This is of interest in view of the coordinated cell activity needed for the control of bone remodelling. Intercellular signal propagation might represent a mechanism by which mechanically initiated signals, possibly from osteocytes, diffuse through the bone tissue to surface osteoblasts and osteoclasts. In a follow-up study, signalling between osteoblasts and osteoclasts was investigated. Surprisingly, signalling to osteoclasts was not mediated by P2Y receptors but appeared to require the $P2X_7$ receptor [42].

The observation that the functionally effective concentrations of ATP and UTP (and ADP) are in the low micromolar range could be relevant to the bone microenvironment, where low-level fluctuations of extracellular nucleotide concentrations are likely to occur. Cultured osteoblasts are capable of releasing ATP, resulting in nanomolar concentrations in the medium [43]. However, concentrations measured in tissue culture medium are unlikely to reflect accurately concentrations occurring at the cell surface and in the small volumes of the extracellular microenvironment in intact tissues. Here, extracellular nucleotide levels might be expected to be in the micromolar range before breakdown by ecto-nucleotidases takes place. So far, release of UTP has been reported for several cell types, although not for osteoblasts. However, UTP could easily be generated extracellularly from other nucleotides through the action of ecto-nucleotidases [44,45]. UTP can also act through P2Y receptors to upregulate ATP release from human osteoblasts, providing a possible positive feedback mechanism [37]. Table 3 summarizes evidence and possible functions of P2 receptors in osteoblasts.

P2 receptors and cartilage

The existence of P2 receptors on chondrocytes was first demonstrated by the work of Russell and colleagues [46,47]. Recent localization studies showed the presence of both P2X and P2Y receptors in chondrocytes: $P2X_2$ and $P2X_5$ receptors were identified by immunohistochemistry, and $P2Y_1$ and $P2Y_2$ receptors were identified by *in situ* hybridization [8] (the latter is in agreement with suggestions made some time ago [46]). First studies of the role of P2 receptors in chondrocytes showed that ATP and ADP, and less strongly UTP, stimulate the production of PGE by cultured human chondrocytes [46], which was enhanced by the pro-inflammatory cytokines interleukin 1β (IL-1β), IL-1α and tumour necrosis factor α (TNF-α) [48–50]. Additionally, extracellular ATP and UTP, but not ADP, have been shown to stimulate cartilage resorption [47,51,52]; again, this was enhanced by simultaneous application of IL-1β and TNF-α. As for osteoblasts, cultured chondrocytes are also capable of constitutively releasing ATP at concentrations in the micromolar range, which might activate P2 receptors in the local microenvironment [53].

Conclusions and therapeutic potential

Taken together, these results indicate that the low-dose effects of extracellular nucleotides on bone resorption and formation are mediated via different P2 receptor subtypes. ADP is a powerful stimulator of osteoclast formation and activity, signalling through the $P2Y_1$ receptor, whereas UTP could play a role as an inhibitor of bone formation, via the $P2Y_2$ receptor, while not affecting osteoclast function.

The effects of nucleotides on osteoclast formation and activity, osteoblast function and osteoclast and osteoblast cell death seem to be restricted to differing concentration ranges (from nanomolar to millimolar for the latter). Thus, it is possible that nucleotides, once released in the bone microenvironment, form concentration gradients by diffusion and degradation, enabling differential targeting of receptors to produce selective spatial effects. A speculative model for the role and interactions of P2 receptors on bone cells is shown in Fig. 2.

Because both ADP and ATP are potent stimulators of bone and cartilage resorption, and ATP and UTP are inhibitors of bone formation, nucleotides (with ATP as the universal agonist) seem to have an overall destructive effect on the skeleton, resulting in net bone loss. Nucleotides could thus present interesting targets for drug developments in the future for several pathological bone loss conditions. This includes osteoporosis, the most common metabolic disorder of the skeleton, which is characterized by low bone mass and disruption of bone architecture as a result of a net excess of bone resorption over bone formation.

The osteolytic activity of ADP could also be relevant to inflammatory conditions such as rheumatoid arthritis that lead to sustained systemic and localized bone loss. To date, most studies suggest that this process is driven by pro-inflammatory, osteolytic prostanoids and cytokines, released from inflamed synovium, such as PGE, IL-6, TNFs and RANKL [54]. However, extracellular nucleotides stimulate cartilage resorption and the production of

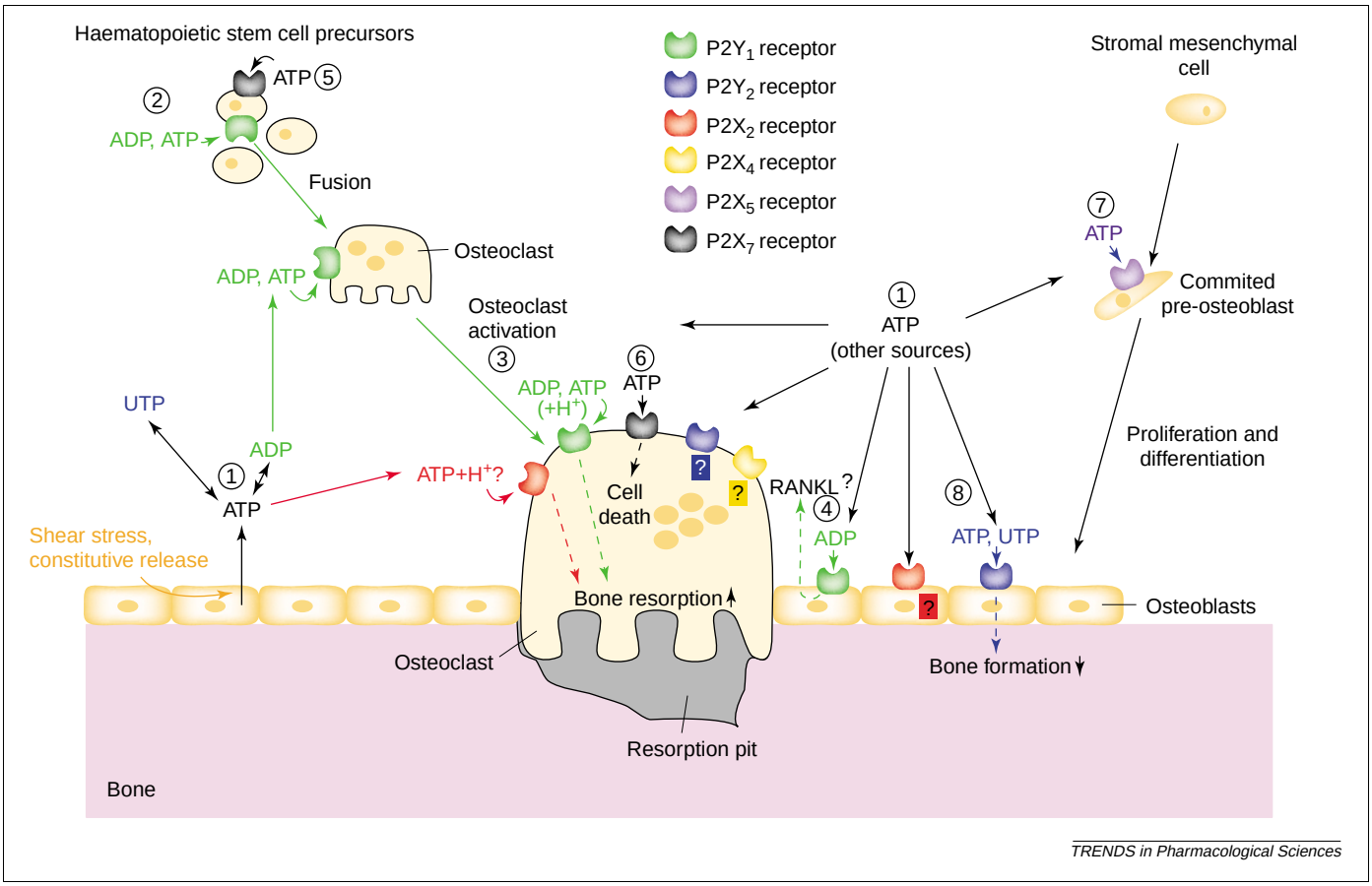

Fig. 2. Schematic diagram illustrating the potential roles played by extracellular nucleotides and P2 receptors in modulating bone cell function. Adenosine 5′-triphosphate (ATP), released from osteoblasts (e.g. through shear stress or constitutively) or from other sources, can be degraded to adenosine 5′-triphosphate (ADP) or converted into uridine 5′-triphosphate (UTP) via ecto-nucleotidases (1). All three nucleotides can act separately on specific P2 receptor subtypes, as indicated by the colour coding. ATP is a universal agonist, whereas UTP is only active at the $P2Y_2$ receptor and ADP is only active at the $P2Y_1$ receptor. ADP, via $P2Y_1$ receptors, appears to stimulate both the formation (i.e. fusion) of osteoclasts from haematopoietic precursors (2) and the resorptive activity of mature osteoclasts (3). For the latter, a synergistic action of ATP and protons has also been proposed via the $P2X_2$ receptor. ADP could also stimulate resorption indirectly through actions on osteoblasts, which in turn release pro-resorptive factors [e.g. receptor activator of nuclear factor κB ligand (RANKL)] (4). ATP at high concentrations might facilitate fusion of osteoclast progenitors through $P2X_7$ receptor pore formation (5) or induce cell death of mature osteoclasts via $P2X_7$ receptors (6). In osteoblasts, ATP, via $P2X_5$ receptors, might enhance proliferation and/or differentiation (7). By contrast, UTP, via $P2Y_2$ receptors, is a strong inhibitor of bone formation by osteoblasts (8). For some receptors (e.g. $P2X_4$ and $P2Y_2$ receptors on osteoclasts or $P2X_2$ receptors on osteoblasts) evidence for expression has been found but their role is still unclear (question marks). Dashed lines indicate signalling events in the cell.

PGE by cultured chondrocytes, an effect enhanced by the pro-inflammatory cytokines IL-1α and IL-1β and TNF-α [50]. Results from mouse calvarial resorption assays showed that ADP and ATP are as powerfully pro-resorptive as is PGE_2 [13,15] and, as discussed above, might additionally act through stimulating PG release. Moreover, release of nucleotides is increased under inflammatory conditions, suggesting an early role of extracellular nucleotides in the inflammatory process. In addition, platelets play a key role in inflammation by being induced to release their granule contents, including adenine nucleotides. Taken together, these observations suggest a mechanism by which nucleotides, perhaps acting in synergism with pro-inflammatory cytokines, could contribute to the pathophysiology of arthritic conditions.

Another pathological condition where ADP-mediated bone resorption could play a major role is the bone loss associated with cancer metastases. Tumour cells are important sources of extracellular ATP [55]. Therefore, localized ATP release (and subsequent breakdown to ADP) could stimulate formation and activation of osteoclasts. Most importantly, inflamed and cancerous tissues are also characterized by low extracellular pH, which would facilitate the osteolytic actions of ADP and ATP.

Outlook

Research over the past decade has revealed that extracellular nucleotides and their receptors might constitute an important and previously unrecognized system for the local regulation of bone cell function. The actions of ATP, the key ligand, on bone appear to be strongly negative, or catabolic, rivalling the impact of the better-known prostanoids and cytokines. However, P2 receptors are widely distributed, not only on bone cells, but also elsewhere in the body. The challenge for the future will be to identify antagonists for the actions of ATP, ADP and UTP that are sufficiently specific for bone, or to devise strategies that limit extracellular levels of these nucleotides in the bone microenvironment.

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