Purinergic signalling and bone remodelling[☆]
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Accumulating evidence suggests that extracellular nucleotides, signalling through P2 receptors, could play an important role in modulating bone cell function. ATP and other nucleotides can stimulate the formation and resorptive activity of osteoclasts (bone-destroying cells) in addition to inhibiting bone mineralisation by osteoblasts. This review discusses the current understanding of the effects of extracellular nucleotides on skeletal cells.

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Introduction

Bone is a composite tissue containing inorganic mineral salts deposited within an organic collagen matrix, and three major cell types: osteoblasts, osteoclasts and osteocytes. Continuous remodelling by bone cells allows the skeleton to grow, adapt and repair itself; abnormalities in this process result in a variety of skeletal disorders.

Osteoblasts, the bone-forming cells, are derived from mesenchymal stem cells. Bone formation is a two-step process, the first stage being synthesis and deposition of the organic matrix. Mature osteoblasts synthesize and release, via exocytosis, type I collagen (85–90% of organic matrix) and many noncollagenous bone matrix proteins (10–15%). The deposited organic matrix (known as osteoid) is subsequently mineralised by calcium and phosphate ions to produce calcified bone tissue; the mineral approximates to hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. In some cases, osteoblasts become incorporated within the bone matrix they secrete and undergo a terminal differentiation to form osteocytes. Within bone, osteocytes form a regular interconnected network of cells that is thought to mediate responses to mechanical loading. Osteoclasts, the bone-resorbing cells, are usually multi-nuclear, and are formed from mononuclear progenitors of the monocyte/macrophage lineage. Following their attachment to bone and activation, osteoclasts undergo a polarisation, forming a sealed compartment that corresponds to an 'extracellular vacuole' over the resorption site. Osteoclasts then secrete protons to dissolve the bone mineral and enzymes (particularly cathepsin K) into this vacuole to degrade the collagenous matrix. Osteoclasts destroy bone rapidly and are normally only present in low number in adult bone.

The concept that purines act as extracellular signalling molecules was first suggested by Drury and Szent-Györygi in 1929, yet it was not until 1972 that the concept of purinergic neurotransmission was proposed [1]. It is now well recognised that extracellular nucleotides, signalling via P2 receptors, participate in a wide number of biological processes in both neuronal and non-neuronal tissues. The receptors for purines and pyrimidines are classified into two groups; P1 receptors, which are primarily activated by adenosine and P2 receptors, which respond to nucleotides including adenosine triphosphate (ATP), adenosine diphosphate (ADP), uridine triphosphate (UTP) and uridine diphosphate (UDP). The P2 receptors are further subdivided into the P2X ligand-gated ion channels and P2Y G-protein-coupled receptors [2,3]. Currently, seven P2X receptors ($P2X_{1-7}$) and eight P2Y receptors ($P2Y_{1,2,4,6,11-14}$) have been identified; each of these receptors has been cloned, characterised and displays distinct tissue expression and pharmacology [4] (Table 1).

Within the field of 'purinergic signalling', the regulation of bone cell function by extracellular nucleotides has emerged as a particularly active and promising area of research. This review will summarize current understanding into the role of extracellular nucleotides and P2 receptors in bone remodelling (Figure 1).

The role of P2 receptors in osteoblast biology
Early work demonstrated that extracellular nucleotides could transiently increase $[Ca^{2+}]_i$ and induce inositol (1,4,5)-trisphosphate formation in osteoblast-like cells [5]; subsequent pharmacological studies indicated the presence of at least two P2 receptor subtypes on osteoblast-like cells [6]. The expression of multiple P2 receptor subtypes by osteoblasts has now been reported

Table 1

P2 receptor pharmacology and expression: summary of published data

Receptor	Main distribution	Agonists	Antagonists	Transduction mechanisms
P2X$_1$	Smooth muscle, platelets, cerebellum, dorsal horn spinal neurons, bone cells	ATP = 2-MeSATP $\geq$ α,β-meATP = β,γ-meATP	TNP-ATP, IP$_5$I, NF023, RO1, NF449	Cation channel (Ca^{2+} and Na^{2+})
P2X$_2$	Smooth muscle, CNS, chromaffin cells, autonomic and sensory ganglia, bone cells	ATP $\geq$ ATPγS $\geq$ 2-MeSATP $\geq$ α,β-meATP (pH and Zn^{2+} sensitive)	Suramin, isoPPADS, NF770, NF279, RB2, NF778	Cation channel (Ca^{2+})
P2X$_3$	Sensory neurones, some sympathetic neurons, bone cells	2-MeSATP $\geq$ ATP $\geq$ α,β-meATP $\geq$ Ap$_5$A	TNP-ATP, PPADS, RO4, NF110, RO51, spinorphin, Ip$_5$I	Cation channel
P2X$_4$	CNS, testis, colon, bone cells	ATP $\gg$ α,β-meATP = 2-MeSATP	TNP-ATP (weak), BBG (weak), phenolphthalein	Cation channel (Ca^{2+})
P2X$_5$	Proliferating cells in skin, gut, bladder, thymus, spinal cord, bone cells	ATP = 2-MeSATP = ATPγS > α,β-meATP	Suramin, PPADS, BBG	Cation channel
P2X$_6$	CNS, motor neurons in spinal cord	Functions poorly as a homomultimer	–	Cation channel
P2X$_7$	Immune cells, pancreas, skin, bone cells	Bz-ATP $\geq$ 2-MeSATP $\geq$ ATP	A348079, KN62, KN04, MRS2427, O-ATP, A-740003, A-804598	Cation channel large pore after prolonged activation
P2Y$_1$	Epithelial and endothelial cells, platelets, immune cells, bone cells	MRS2365 > 2-MeSADP = ADPβS > 2-MeSATP = ADP > ATP	MRS2179, MRS2279, MRS2279	G$_q$/G$_{11}$ PLCβ activation
P2Y$_2$	Immune cells, epithelial and endothelial cells, kidney tubules, bone cells	2-thio-UTP > UTP = ATP > UTPγS	Suramin > RB2	G$_q$/G$_{11}$, possibly G$_i$/G$_o$ PLCβ activation
P2Y$_4$	Endothelial cells, osteoblasts	UTP > ATP > Up$_4$U > UTPγS	RB2 > suramin	G$_q$/G$_{11}$, possibly G$_i$ PLCβ activation
P2Y$_6$	Some epithelial cells, placenta, T cells, thymus, bone cells	3-Phenylacyl UDP > UDPβS > UDP > UTP $\gg$ ATP	MRS2578	G$_q$/G$_{11}$ PLCβ activation
P2Y$_{11}$	Spleen, intestine, granulocytes	AR-C67085MX > Bz-ATP = ATPγS > ATP	Suramin > RB2, NF157	G$_q$/G$_{11}$ and G$_s$ PLCβ activation
P2Y$_{12}$	Platelets, glial cells, bone cells	2-MeSATP $\geq$ 2-MeSADP > ADP > ATP	CT50547, ARL66096, clopidogrel	Gα_i; inhibition of adenylate cyclase
P2Y$_{13}$	Spleen, brain, lymph nodes, bone marrow	ADP = 2-MeSADP $\gg$ 2-MeSATP > ATP	MRS2211, 2-MeSAMP	G$_i$/G$_o$
P2Y$_{14}$	Placenta, adipose tissue, stomach, intestine, bone cells	UDP-glucose $\geq$ UDP-galactose	–	G$_q$/G$_{11}$

Table modified from Burnstock [64].

Figure 1
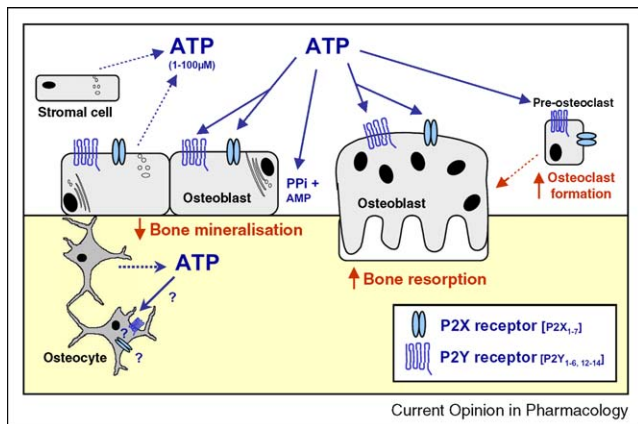
Overview of the known functional effects of ATP on bone cells. ATP released from osteoblasts, stromal cells and osteocytes can act locally to inhibit bone mineralisation and stimulate osteoclast formation and activity.

A growing body of work indicates that extracellular nucleotides, signalling via P2 receptors, could play a role in modulating osteoblast function (Table 3). ATP acting via the $P2X_5$ receptor has been reported to stimulate osteoblast proliferation [8], whilst activation of the $P2Y_1$ receptor is thought to modulate osteoblast responses to systemic factors such as parathyroid hormone [15,16]. P2Y receptor stimulation by ATP has also been associated with increased interleukin-6 synthesis [12].

The role of the $P2X_7$ receptor in osteoblast function is less clear (for a detailed review on the $P2X_7$ receptor see [17••]). Early reports suggested that $P2X_7$ receptor activation caused enhanced osteoblast apoptosis [9]. In contrast, more recent studies have suggested that $P2X_7$ stimulation leads to increased membrane blebbing and bone formation; an effect thought to be mediated via increased production of lypophosphatidic acid (LPA) and prostaglandin E_2 (PGE_2) [18–20]. The $P2X_7$ receptor is also thought to mediate the ERK1/2 activation caused by fluid shear stress in osteoblast-like cells [21].

by a number of groups (Table 2) [7–10,11•,12–14] (IR Orriss, abstract in *Bone* 2009, 44:S304). Furthermore, recent studies have demonstrated that P2 receptor expression in osteoblasts is strongly differentiation-dependent [13].

The first study of the effects of extracellular nucleotides on bone formation *in vitro* by cultured primary osteoblast showed that ATP and UTP were strongly inhibitory at concentrations $\geq 1 \mu M$ [22]. A follow-up investigation demonstrated that ATP-treated and UTP-treated osteoblasts deposited abundant collagenous matrix with the

Table 2

P2 receptor expression by osteoblasts

Receptor	Species	Cell type	Evidence for expression	References
$P2X_1$	Rat	Primary	qPCR, ICC	(IR Orriss, abstract in *Bone* 2009, 44:S304)
$P2X_2$	Rat	Primary	ISH, RT-PCR, ICC	[10,13]
	Human	MC3T3-E1	RT-PCR	[8]
	Human	MG-63 and SaOS-2	RT-PCR	[14]
$P2X_3$	Rat	Primary	qPCR, ICC	(IR Orriss, abstract in *Bone* 2009, 44:S304)
$P2X_4$	Rat	Primary	qPCR, ICC	(IR Orriss, abstract in *Bone* 2009, 44:S304)
	Human	SAM-1	RT-PCR	[12]
	Human	MG-63 and SaOS-2	RT-PCR	[14]
$P2X_5$	Rat	Primary	RT-PCR, ICC	[10,13]
	Human	MC3T3-E1	RT-PCR	[8]
	Human	SAM-1	RT-PCR	[12]
$P2X_6$	Rat	Primary	qPCR, ICC	(IR Orriss, abstract in *Bone* 2009, 44:S304)
	Human	SAM-1	RT-PCR	[12]
$P2X_7$	Rat	Primary	RT-PCR, ICC	[13]
	Human	MG-63	RT-PCR	[8]
	Human	MG-63 and SaOS-2	RT-PCR, ICC, WB	[14]
	Human	Primary and SaOS-2	RT-PCR, ICC	[9]
	Mouse	Primary	RT-PCR	[11•]
$P2Y_1$	Rat	Primary	ISH, RT-PCR, ICC	[10,13]
	Human	MG-63	RT-PCR	[7]
$P2Y_2$	Rat	Primary	ISH, RT-PCR, ICC, WB	[10,13]
	Human	MG-63	RT-PCR	[7]
	Human	Primary, SaOS-2 and Te85	RT-PCR, SB	[37]
$P2Y_4$	Rat	Primary	RT-PCR, ICC, WB	[13]
	Human	MG-63	RT-PCR	[7]

Table 2 (*Continued*)

Receptor	Species	Cell type	Evidence for expression	References
P2Y$_6$	Rat	Primary	RT-PCR, ICC	[13]
	Human	MG-63	RT-PCR	[7]
P2Y$_{12}$	Rat	Primary	qPCR, WB	(IR Orriss, abstract in *Bone* 2009, 44:S304)
P2Y$_{13}$	Rat	Primary	qPCR	(IR Orriss, abstract in *Bone* 2009, 44:S304)
P2Y$_{14}$	Rat	Primary	qPCR, WB	(IR Orriss, abstract in *Bone* 2009, 44:S304)

Quantitative real time polymerase chain reaction (**qPCR**), immunocytochemistry (**ICC**), *in situ* hybridisation (**ISH**), reverse transcriptase polymerase chain reaction (**RT-PCR**), western blot (**WB**), southern blot (**SB**).

characteristic morphology of bone nodules, but that mineralisation had failed to occur (Figure 2) [23••]. The potent inhibitory actions of ATP and UTP were consistent pharmacologically with mediation via the P2Y$_2$ or P2Y$_4$ receptor subtypes. Reactive blue 2, a P2Y$_4$ receptor antagonist, failed to prevent the nucleotide-induced block of mineralisation, suggesting that P2Y$_2$ receptor stimulation mediates the functional effects of ATP and UTP [23••]. Skeletal analysis of P2Y$_2$ knockout mice by dual energy X-ray absorbtiometry and micro-CT demonstrated striking increases in trabecular and cortical bone parameters in both the femora and tibae [23••] (IR Orriss, abstract in *Calcif Tissue Int* 2008, 83:2–3). In addition, several studies have demonstrated that P2Y$_2$

activation in osteoblast-like cells activates a number of intracellular signalling pathways including protein kinase C (PKC), p38 mitogen-activated protein kinase (p38 MAPK) and c-Jun NH$_2$-terminal protein kinase (JNK) [24–27].

ATP is present in the cytoplasm of mammalian cells at concentrations between 2 and 5 mM. Following membrane damage or necrosis, all cells can potentially release ATP into the extracellular environment, which can then act in an autocrine/paracrine manner to influence local purinergic signalling. Controlled ATP release has been demonstrated from numerous excitatory and nonexcitatory cells including osteoblasts [28,29•,30,31]. ATP

Table 3

P2 receptors and bone cell function

Receptor	Proposed function	Signalling	References
Osteoblasts			
P2X$_5$	Increased osteoblast proliferation	Stimulation of the MAP kinase pathway	[8]
P2X$_7$	Induction of osteoblast apoptosis		[9]
	Induction of membrane blebbing and increased bone formation	Activation of PLD and PLA$_2$ stimulates LPA and PGE$_2$ synthesis/release	[18–20]
	Fluid shear stress induced activation of ERK1/2	Increased [Ca^{2+}]$_i$ and PKC activation	[21,65]
P2Y$_1$	Modulate osteoblast responses to systemic factors e.g. PTH	Increased c-fos expression	[15,16]
P2Y$_2$	Propagation of intercellular Ca^{2+} waves	–	[66]
	Inhibition of bone mineralisation	Inhibition of ALP	[22,23••]
	Stimulation of Erg1 and Runx2 expression	Activation of the PKC and ERK pathways	[25,67]
	Sensitises mechanical stress-activated Ca^{2+} channels	Activation of ERK, p38 MAPK and JNK1 pathways	[26,27]
P2Y	Increased Il-6 expression	–	[12]
Osteoclasts			
P2X$_2$	Increased bone resorption	–	[43]
P2X$_7$	Increased apoptosis	–	[51]
	Intercellular communication	–	[68]
	Precursor cell fusion	–	[47]
	Regulation of osteoclast formation and activity	Translocation and activation of NF$_K$B	[49]
	Decreased apoptosis	Inhibition of caspase-3	[48]
	?	PKCα translocation to the basolateral membrane	[50]
	Cytoskeletal reorganisation and the delivery and secretion of lytic granules	Activation of the Syk pathway	[52]
P2Y$_1$	Increased osteoclast formation and bone resorption	–	[44]
P2Y$_6$	Increased osteoclast survival	Translocation and activation of NF$_K$B	[41]

Phospholipase D (**PLD**), phospholipase A$_2$ (**PLA$_2$**), lypophosphatidic acid (**LPA**), prostaglandin E$_2$ (**PGE$_2$**), protein kinase C (**PKC**), alkaline phosphatase (**ALP**), extracellular related kinase (**ERK**), p38 mitogen-activated protein kinase (**p38 MAPK**), c-jun NH$_2$-terminal kinase 1 (**JNK1**), nuclear factor kappa-light-chain-enhancer of activated B cells (**NF$_K$B**).
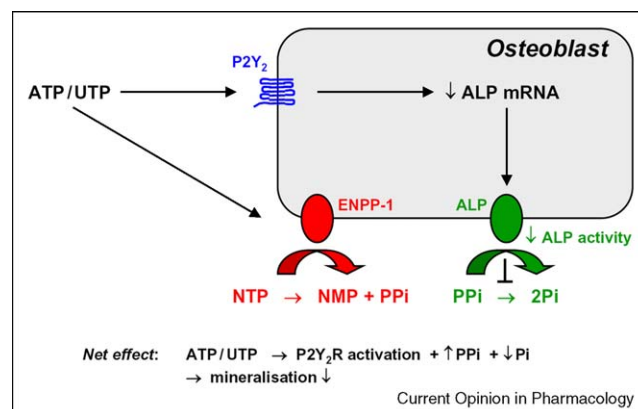
Figure 2
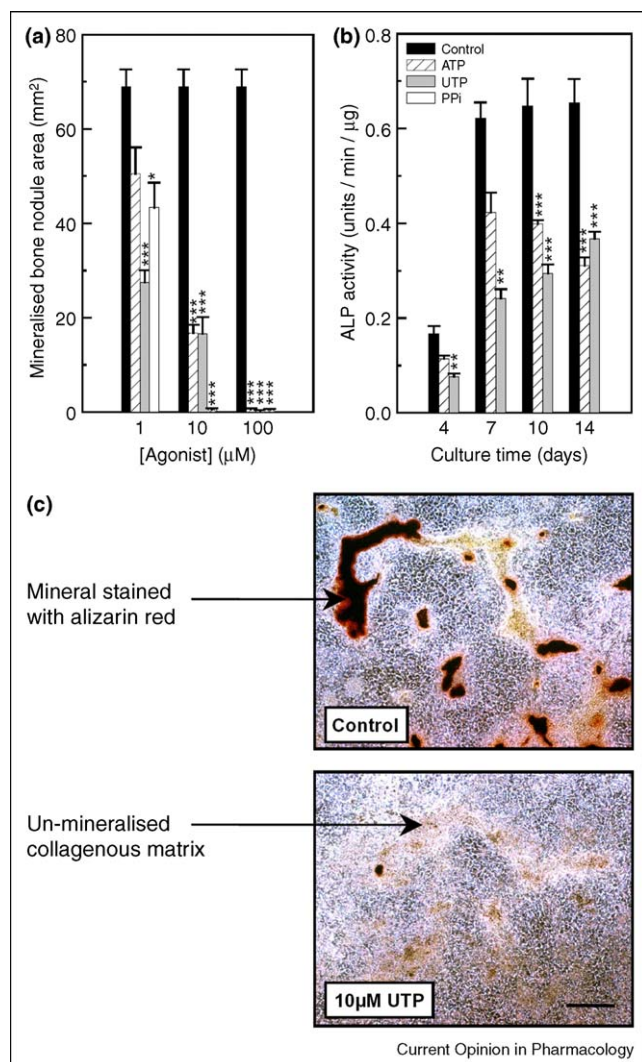
ATP and UTP inhibit bone mineralisation *in vitro*. **(a)** ATP, UTP and PPi (≥ 1 μM) inhibit mineralised bone nodule formation by rat osteoblasts. **(b)** ATP and UTP (10 μM) inhibit osteoblast alkaline phosphatase activity (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$). The images in **(c)** show *in vitro* 'trabecular-shaped' bone nodule formation by rat osteoblasts under normal conditions and the striking inhibition of mineralisation in osteoblasts treated with UTP.

Figure 3

The effect of ATP and UTP on extracellular PPi and mineralisation. Schematic diagram of the potential mechanism by which extracellular nucleotides inhibit bone mineralisation. ATP and UTP, acting via the P2Y$_2$ receptor (and possibly also the P2Y$_4$ receptor) on mature, bone-forming osteoblasts, cause decreased expression/activity of alkaline phosphatase (ALP). This in turn would lead to an increase in extracellular pyrophosphate (PPi, a key physiological inhibitor of mineralisation) and a decrease in local Pi levels. Concurrently, nucleotide triphosphates (NTP) such as ATP and UTP can also be hydrolysed by osteoblast ecto-pyrophosphatase/phosphodiesterase-1 (E-NPP1) to generate PPi directly. The combined effect is a net increase in extracellular PPi concentration, leading to a decrease in mineralisation.

release from osteoblasts *in vitro* has been reported in the range 0.5–1 nmol/ml under normal conditions [31]. Since osteoblasts *in vivo* are bathed by considerably lower volumes of extracellular fluid than is the case *in vitro*, these data suggest the possibility that local concentrations of ATP *in vivo* could be considerably higher, in the range 1–100 μM [31]. ATP release is enhanced by a number of external stimuli including fluid shear stress [30], hypoxia [31] and vitamin D$_3$ [32]. Increased ATP release in response to shear stress has been associated with mechanotransduction since P2 receptor activation by secreted ATP mediates fluid-flow induced PGE$_2$ release [30].

Once released, nucleotides are rapidly broken down by an extracellular hydrolysis cascade. Molecular and functional characterisation has shown there are four families of ecto-nucleotidases: firstly, the E-NTPdases (ecto-nucleoside triphosphate diphosphohydrolase); secondly, the E-NPPs (ecto-nucleotide pyrophosphatase/phosphodiesterase); thirdly, alkaline phosphatases; and fourthly, ecto-5′nucleotidase [33•]. Many ecto-nucleotidases have overlapping specificities. For example, E-NTPdases catalyse the reactions: NTP → NDP + phosphate (Pi) and NDP → NMP + Pi, whereas E-NPPs hydrolyse NTP → NMP + pyrophosphate (PPi) or NDP → NMP + Pi. Thus, the combined activity of these ecto-enzymes will tend to limit the actions of extracellular nucleotides to cells within close proximity of the release site. Osteoblasts express three members of the E-NPP family, E-NPP1, E-NPP2 and E-NPP3 [23••,34]. A recent study demonstrated that osteoblastic E-NPP activity was capable of generating significant concentrations of PPi *in vitro* [23••]. Since PPi is a potent inhibitor of bone mineralisation [35], it is likely that nucleotide triphosphates exert a dual inhibitory action on bone mineralisation via both P2 receptor mediated signalling and direct hydrolysis to PPi [23••] (Figure 3).

P2 receptors and osteocytes
Osteocytes are the most abundant cell type in bone, yet the role of purinergic signalling in their survival and function is unknown. Normal osteocytes are difficult to

Table 4

P2 receptor expression by osteoclasts

Receptor	Species	Evidence for expression	References
P2X$_1$	Human[a]	RT-PCR	[39]
	Mouse[b]	qPCR	(IR Orriss, abstract in *Bone* 2009, 44:S304)
P2X$_2$	Rat[c]	ICC, ICH	[10]
	Mouse[b]	qPCR	(IR Orriss, abstract in *Bone* 2009, 44:S304)
P2X$_3$	Mouse[b]	qPCR	(IR Orriss, abstract in *Bone* 2009, 44:S304)
P2X$_4$	Rat[c]	ICC, ICH	[10]
	Mouse[b]	qPCR	(IR Orriss, abstract in *Bone* 2009, 44:S304)
	Rabbit[b]	RT-PCR	[38]
	Human[a]	RT-PCR	[39]
P2X$_5$	Mouse[b]	qPCR	(IR Orriss, abstract in *Bone* 2009, 44:S304)
P2X$_7$	Rat[c]	ICC	[10]
	Mouse[b]	RT-PCR, ICC	[11•,20]
	Human[a]	RT-PCR, ICC	[39,47]
P2Y$_1$	Rat[c]	ISH	[10]
	Mouse[b]	qPCR	(IR Orriss, abstract in *Bone* 2009, 44:S304)
	Human[a]	RT-PCR	[39]
P2Y$_2$	Rat[c]	ISH	[10]
	Mouse[b]	qPCR	(IR Orriss, abstract in *Bone* 2009, 44:S304)
	Human[d]	RT-PCR	[37]
	Human[a]	RT-PCR	[39]
P2Y$_4$	Human[a]	RT-PCR	[39]
P2Y$_6$	Mouse[b]	qPCR	(IR Orriss, abstract in *Bone* 2009, 44:S304)
	Rabbit[c]	RT-PCR	[41]
	Human[a]	RT-PCR	[39]
P2Y$_{11}$	Human[a]	RT-PCR	[39]
P2Y$_{12}$	Mouse[b]	qPCR	(IR Orriss, abstract in *Bone* 2009, 44:S304)
P2Y$_{13}$	Mouse[b]	qPCR	(IR Orriss, abstract in *Bone* 2009, 44:S304)
P2Y$_{14}$	Mouse[b]	qPCR	(IR Orriss, abstract in *Bone* 2009, 44:S304)

Quantitative real time polymerase chain reaction (**qPCR**), immunocytochemistry (**ICC**), *in situ* hybridisation (**ISH**), reverse transcriptase polymerase chain reaction (**RT-PCR**).
[a] Osteoclasts derived from peripheral blood monocytes.
[b] Osteoclasts derived from the bone marrow or spleen.
[c] Osteoclasts isolated from the long bones.
[d] Osteoclastoma.

study *in situ*, owing to their location within the mineralised bone matrix, and cannot easily be isolated for primary cell culture. To date P2 receptor expression by osteocytes has not been reported; although since mature osteoblasts express multiple P2 receptor subtypes [13], purinergic receptor expression by osteocytes seems likely. A recent study demonstrated that cultured MLO-Y4 osteocyte-like cells release ATP in response to shear stress [36•]; there is no other published information available regarding ATP release from osteocytes. An intriguing possibility is that ATP released from osteocytes entombed in bone might help to prevent cell mineralisation (and thus death).

The role of P2 receptors in osteoclast biology

Studies in a number of laboratories have indicated that osteoclasts express multiple P2 receptors (Table 4) [10,11•,20,37–41] (IR Orriss, abstract in *Bone* 2009, 44:S304). A role for the P2 receptors in the regulation of osteoclast function was first suggested in 1995 by Bowler *et al.* [37] after ATP was found to stimulate resorption by cells derived from human osteoclastoma. Initially, this effect was thought to be mediated via the P2Y$_2$ receptor; however, in a follow-up study, UTP failed to stimulate bone resorption [42], suggesting this was not the case. Subsequently, ATP was found to stimulate the formation and activation of rodent osteoclasts; the resorptive activity being further increased when osteoclasts were first activated by culture in acidified medium [43]. These pro-resorptive effects were suggested to involve the P2X$_2$ receptor since it is the only P2 receptor sensitive to protons. Further investigation showed that low micromolar concentrations of ATP, ADP and 2-MeSADP potently stimulated both the formation and

resorptive activity of rodent osteoclasts [44]. These observations, combined with cytochemical evidence, suggest involvement of the $P2Y_1$ receptor in mediating the osteolytic effects of ATP and ADP [44,45]. Conversely, a study on human osteoclasts suggested that ATP functions indirectly, via upregulation of RANKL on osteoblasts, to stimulate resorption [39] (Table 3).

The role of the $P2X_7$ receptor, polymorphisms of which are associated with fracture risk in postmenopausal women [46••], in osteoclast formation and activity appears complex (Table 3). Initial experiments using cells derived from human peripheral blood demonstrated that $P2X_7$ receptor antagonism inhibited osteoclast formation [47], suggesting a potential role in cell fusion. Analogues of the $P2X_7$ receptor antagonist, KN-62, have also been shown to induce osteoclast apoptosis [48]. In contrast, a report by Ke *et al.* [11•] demonstrated that $P2X_7$-deficient mice possessed functional osteoclasts *in vivo*. Furthermore, using knockout precursor cells, osteoclasts could be generated *in vitro*, indicating that the $P2X_7$ receptor is not required for cell fusion [11•]. Activation of the $P2X_7$ receptor has also been shown to induce the translocation and activation of NF_KB (nuclear factor kappa-light-chain-enhancer of activated B cells) [49] and PKC [50] in osteoclasts and their precursors. Additionally, the $P2X_7$ receptor may also play a role in intercellular communication between bone cells [51], cytoskeletal reorganisation at the sealing zone and the delivery and secretion of lytic granules into the resorption lacunae [52].

The $P2Y_6$ receptor has been suggested to play a role in osteoclast survival since the activation of this receptor prevented the apoptosis induced by $TNF\alpha$ [41]. Furthermore, stimulation of the $P2Y_6$ receptor by UDP induced the translocation and activation of NF_KB in osteoclasts and their precursors [41].

P2 receptors and cartilage
P2 receptor expression in cartilage was first suggested in 1991 when ATP was shown to stimulate PGE_2 production from articular chondrocytes [53]. Expression of multiple P2 receptor subtypes [10,54–56] and constitutive ATP release [57] from chondrocytes has now been reported. Available data regarding the effects of purinergic signalling on cartilage are conflicting. Some studies suggest extracellular nucleotides negatively regulate cartilage metabolism, since ATP reportedly inhibits cartilage formation in chick limb bud micromass cultures [58], promotes proteoglycan breakdown and glycosaminoglycan release [59] and increases the production of the inflammatory mediators, nitric oxide (NO) and PGE_2 [60]. In contrast, reported beneficial effects of ATP on cartilage metabolism include upregulation of proteoglycan synthesis and collagen accumulation [61] and suppression of inflammatory mediator (NO) production [62]. In addition, ATP signalling via the $P2X_4$ receptor is thought

to mediate the increased intracellular Ca^{2+} required for chondrocyte differentiation [63•]. Our own unpublished data indicate that ATP causes a dose-dependent stimulation of the formation of chondrocytic nodules in chick micromass cultures.

Conclusions and future directions
The ATP-P2 receptor signalling system can exert complex local effects on the function of skeletal cells. The results summarised here suggest that the main functional impact of extracellular nucleotides on bone may be negative, with effects on osteoblast function being particularly notable. Selective receptor agonists and antagonists for the P2 receptor subtypes involved in bone remodelling are currently being developed, which hopefully will lead to novel therapeutic strategies to treat bone disease.

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