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Transcriptional Control Mechanisms Associated with the Nucleotide Receptor P2X₇, a Critical Regulator of Immunologic, Osteogenic and Neurologic Functions

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Abstract

The nucleotide receptor P2X₇ is an attractive therapeutic target and potential biomarker for multiple inflammatory and neurologic disorders, and it is expressed in several immune, osteogenic and neurologic cell types. Aside from its role in the nervous system, it is activated by ATP released at sites of tissue damage, inflammation and infection. Ligand binding to P2X₇ stimulates many cell responses, including calcium fluxes, MAPK activation, inflammatory mediator release, and apoptosis. Much work has centered on P2X₇ action in cell death and mediator processing (e.g., pro-interleukin-1 cleavage by the inflammasome), but the contribution of P2X₇ to transcriptional regulation is less well defined. In this review, we will focus on the growing evidence for the importance of nucleotide-mediated gene expression, we will highlight several animal model, human genetic, and clinical studies that support P2X₇ as a therapeutic target, and we will discuss the latest developments in anti-P2X₇ clinical trials.

Keywords

Nucleotide Receptors; P2X₇; Gene Transcription; FosB; Macrophages; Inflammation

Introduction

Extracellular nucleotides serve as important signals in a variety of biological processes, including inflammation, tissue repair, bone remodeling, apoptosis and neurotransmission, and dysregulation of nucleotide-mediated signaling contributes to altered physiological responses and disease states (1, 2). In this regard, multiple nucleotides (i.e., ATP, ADP, UTP, UDP, etc.) can be released at sites of tissue injury, infection, platelet activation, mechanical stimulation, and in tumor microenvironments, and these molecules can bind to the P2 family of nucleotide receptors to stimulate a plethora of downstream events (3, 4). The levels of nucleotides in the extracellular space are normally low and are regulated by ectonucleotidases. However, in the event of localized trauma, high concentrations of nucleotides capable of activating the P2 receptors are achieved when damaged cells either release nucleotides and/or their ectonucleotidases are down-regulated (5). The P2 family is subdivided into the P2X ionotropic receptors (P2X₁₋₇) and the P2Y metabotropic family members (P2Y₁, P2Y₂, P2Y₄, P2Y₆, P2Y₁₁₋₁₄). The P2X receptors are ion-gated calcium channels, and the P2Y receptors are seven transmembrane-spanning heterotrimeric G-protein coupled receptors (6, 7). The P2X and P2Y family members are expressed in numerous cell types and function in diverse processes including apoptosis, inflammatory

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mediator production, and platelet activation. Antagonists for P2X and P2Y receptors are being developed to treat numerous diseases, such as atherothrombosis, rheumatoid arthritis, neurodegenerative diseases and pain, by attenuating extracellular nucleotide-induced actions (8–12). Therefore, understanding the mechanisms by which P2 receptors function in both normal and disease states is critical for developing therapies that target these receptors.

One nucleotide receptor that is gaining interest as a potential therapeutic target and biomarker for an array of inflammatory, pathogenic, osteogenic and psychological diseases is P2X₇. The P2X₇ nucleotide receptor is expressed in several cell types, including monocytes, macrophages, osteoblasts, osteoclasts, astrocytes and microglia (13–18). Activation of P2X₇ by ATP stimulates multiple signaling processes, including ion fluxes (Ca²⁺ and Na⁺ influx, K⁺ efflux), the mitogen-activated protein kinases (MAPKs) ERK1/2, p38 and JNKs, the NADPH oxidase complex and reactive oxygen species (ROS) formation, phospholipase D, several caspases, and the formation of a non-specific pore permeable to small molecules (<900 Da). P2X₇ enhances lipopolysaccharide (LPS)-initiated mediator production and can directly augment the expression, processing and/or secretion of many immuno-modulatory factors, such as interleukin-1 β (IL-1 β), IL-8, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and vascular endothelial growth factor (VEGF) (12, 19–22). Because P2X₇ action appears to be involved in the synthesis and release of multiple inflammatory mediators, it has been hypothesized that the production of a pharmacological inhibitor against P2X₇ will reduce the magnitude of an inflammatory response under physiological conditions where an excessive immune reaction is unfavorable. In this regard, there are P2X₇ antagonists in clinical trials for rheumatoid arthritis, and recent developments concerning anti-P2X₇ therapies will be discussed below under **“P2X₇ As a Potential Therapeutic Target and Biomarker”**.

The human P2X₇ receptor is comprised of 595 amino acids, and numerous polymorphisms in its promoter, introns and exons have been identified, including several non-synonymous single nucleotide polymorphisms (SNPs) with links to disease (6, 23, 24). As illustrated in Fig. 1, P2X₇ is predicted to possess a short intracellular N-terminal domain, two transmembrane domains, a large extracellular ligand-binding domain, and an intracellular C-terminal domain that is important for lipid binding and for proper plasma membrane expression of the protein (23, 25, 26). The receptor contains five N-linked glycosylation sites, and we have shown that glycosylation at N187 is critical for normal function and for receptor expression on the cell surface (13). Residue N187 is located near two SNPs (E186K and L191P) that have been reported to lead to attenuated P2X₇ function (27). In addition, P2X₇ contains intracellular palmitoylation sites that are important for its association with detergent-resistant membranes (28). Interestingly, P2X₇ is unique from the other P2X family members because its C-terminal tail is at least 100 amino acids longer than the other P2X receptors, and P2X₇ is one of two P2X receptors reported to promote the formation of a non-specific pore (6, 7, 23, 29). Prolonged activation of P2X₇ results in the formation of the reversible pore, leading to the passage of small molecules (<900 Da), and in certain circumstances, pore formation has been associated with the induction of apoptosis (12). In contrast, transient stimulation with P2X₇ ligands, which may reflect a more physiological scenario given the rapid degradation of nucleotides in the extracellular environment, has been reported to lead to alterations in gene transcription rather than cell death (20, 30).

In this review, we will highlight several of the recent reports that have demonstrated P2X₇ may be an effective therapeutic target and biomarker for numerous inflammatory and neurological diseases by examining key animal model, human genetic, and clinical studies. We will also discuss the evidence that P2X₇ can promote gene expression upon transient activation, spotlight our recent finding that P2X₇ regulates gene transcription by inducing the expression of the activating protein-1 (AP-1) transcription factors FosB and Δ FosB, and

consider the potential significance of the relationship between P2X₇ and FosB/ΔFosB to immunological, osteogenic and neurologic disorders.

P2X₇ As a Potential Therapeutic Target and Biomarker

The recent upsurge in P2X₇ human genetic and animal studies has provided support to the idea that P2X₇ represents a therapeutic target and biomarker for several diseases, including arthritis, asthma, papillary thyroid cancer, spinal cord injury, tuberculosis and mood disorders (8, 31–38). Several reports have correlated P2X₇ SNPs to human disease, and many groups have performed P2X₇ knockout or inhibitor studies in rodents to demonstrate the importance of P2X₇ in immune, osteogenic and neurological regulation.

P2X₇ Knockout Studies

Mice in which the P2X₇ gene has been knocked out have been useful for the study of this receptor because these animals are viable and fertile, and upon initial examination, they do not appear to display any obvious physical or behavioral abnormalities (22, 39, 40). However, upon more detailed physical examination, P2X₇ knockout mice have been found to present with skeletal deformities; specifically, these animals display excessive trabecular bone resorption and deficient periosteal bone formation (39). Examination of the skeletal system of P2X₇-null mice also revealed that stimulation of periosteal bone growth by mechanical loading is markedly attenuated in these animals in comparison to their wild-type littermates (40). It should be noted that mechanical stimulation of osteoblasts (the bone-forming cells of the skeletal system) has been linked to ATP release (41, 42), and P2X₇ expression has been detected in both osteoblasts and osteoclasts (the cells responsible for bone resorption) (2). These skeletal observations will be discussed later in this review.

P2X₇ knockout mice have been subjected to diverse immune challenges and psychological tests to identify additional phenotypic differences between P2X₇ null animals and their wild-type counterpart controls, and these studies have provided several exciting results. A few selected studies utilizing P2X₇ knockout mice will be discussed below; in particular, we will focus on reports pertaining to lung inflammation, pain, glomerulonephritis and depression (43–52):

Using a cigarette smoke-induced lung inflammation mouse model, Lucattelli *et al.* tested the idea that P2X₇ mediates lung inflammation (44). The premise for the study was based on data showing that the concentration of ATP (a P2X₇ ligand) is higher in the lungs of smokers versus non-smokers (43). The authors also found that cigarette smoke increases P2X₇ mRNA expression in alveolar macrophages and neutrophils, and mice treated with the P2X₇ antagonist 1-[N, O-bis(5-isoquinolinesulfonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine (KN-62) and P2X₇ knockout mice are protected from smoke-induced lung inflammation (44). With respect to pain research, several reports have shown that P2X₇ is involved in promoting inflammatory and neuropathic pain (45, 46). Using various models to examine pain, P2X₇ knockout mice and other rodents treated with P2X₇ antagonists were found to experience reduced allodynia and hyperalgesia in comparison to control animals (45, 46). Chessell *et al.* also provided intriguing preliminary data related to P2X₇ function and pain (45). In this study, peripheral nerves proximal to a patient's site of injury were extracted during surgery, and the control nerves were obtained during limb amputations that were unrelated to a neurological issue (45). It was observed that P2X₇ protein expression is higher in extracts from human injured nerves that cause pain in comparison to control nerves (45). With respect to renal disease, Taylor *et al.* examined P2X₇ knockout mice in an immune mediated-glomerulonephritis model and reported that glomerular thrombosis is decreased in the knockout animals when compared to wild-type controls (47). Glomerulonephritis can be an acute or chronic problem and is a common cause of end-stage

kidney disease. Taylor *et al.* observed that macrophage infiltration into glomeruli is decreased in P2X₇ knockout mice and that the P2X₇ inhibitor A-438079 decreases glomerulonephritis in rats (47). Thus, modulation of P2X₇ status may serve as an effective therapeutic approach in several settings.

Although the majority of P2X₇ studies involve an examination of receptor function in inflammation, several groups have provided evidence concerning the potential importance of this receptor from a psychiatric perspective. A dual role for P2X₇ in both inflammation and psychiatry is not unexpected given the intimate interplay between immunological dysfunction and affective disorders (48). For example, depression often coincides with elevated levels of pro-inflammatory cytokines including factors that are regulated by P2X₇ (e.g., IL-1 β , TNF- α , IL-6), it has a high prevalence in individuals who have infectious or autoimmune diseases, and pro-inflammatory cytokines may produce “sickness behavior”, which is a set of adaptive behavioral changes that occur during an infection to help the organism cope with their disease (49–51). Because IL-1 β , which is a well-established target of P2X₇ action, can induce behavioral changes that are similar to depression, Basso *et al.* hypothesized that P2X₇ can promote depressant symptoms (52). In these studies, P2X₇ knockout mice were subjected to two well-characterized tests for depression-like behaviors, namely tail suspension and forced swim tests. In these assessments, the time an animal remains immobile during an inescapable stressor is recorded and is indicative of depressive behavior. Basso *et al.* observed that P2X₇ knockout mice were significantly more mobile than the wild-type controls in both tests, suggesting that attenuation of P2X₇ is associated with anti-depressive behavior (52). Further research on the role of P2X₇ in the context of mood disorders appears warranted given the connection between cytokine production and neurological disorders, as well as recent genetic analyses linking P2X₇ SNPs to mood disorders (27, 52, see below).

P2X₇ Polymorphisms

To date, there are over twenty non-synonymous P2X₇ SNPs that have been entered in the National Center for Biotechnology Information (NCBI) database. Information concerning the location of these SNPs within the P2X₇ protein, their correlation with human disease, and relevant functional data are summarized in Fig. 1 and Table 1. The P2X₇ non-synonymous SNPs identified thus far are located throughout the protein, including the predicted extracellular domain, the second transmembrane domain, and the intracellular C-terminal domain. The two most widely studied P2X₇ SNPs are E496A and Q460R, and both of these polymorphisms are located in the intracellular C-terminal domain. The E496A polymorphism has been correlated with susceptibility to tuberculosis in several populations, and homozygosity of this polymorphism reportedly correlates with papillary thyroid cancer (32, 53–57). Mutation of P2X₇ E496 to Ala results in attenuated ligand-stimulated pore formation, but conflicting data exists about whether it maintains normal cation channel activity (58–60). However, it has been observed that monocytes from subjects with the E496A polymorphism release significantly less IL-1 β and IL-18, which are key immunomodulatory factors that are regulated by P2X₇ (61). Of note, we have detected that the E496A polymorphism exhibits attenuated P2X₇ ligand-stimulated induction of the AP-1 transcription factors FosB and Δ FosB (unpublished observations). Thus, these results are the first to suggest that a P2X₇ polymorphism with a known linkage to human disease confers altered regulation of transcription factors and potentially the expression of other genes.

The P2X₇ Q460R SNP has been examined in the context of mood disorders, and a correlation with bipolar and major depression disorders has been reported in some studies, whereas others have shown no statistically significant associations (62–65). Also, it is currently unclear whether the Q460R polymorphism results in altered ion channel or pore functions. For example, in one study it was reported that the Q460R mutation does not

exhibit altered channel or pore function, whereas it was reported in another study that monocytes from patients with the Q460R polymorphism display enhanced pore function (27, 66). In addition to the E496A and Q460R SNPs, there is one report demonstrating that the A348T polymorphism located within the putative second transmembrane domain is protective against *T. gondii* infection (67). This P2X₇ A348T polymorphism reportedly exhibits enhanced ion channel and pore activity (27). In sum, although it is evident that more studies are needed to determine if P2X₇ polymorphisms may be used as clinical markers for disease risk, the potential exists for P2X₇ to be used as a novel biomarker for diverse diseases.

Denlinger *et al.* have reported that P2X₇ loss-of-function polymorphisms correlate with the likelihood of experiencing an asthma exacerbation following viral infection (31). Although the mechanisms by which these P2X₇ SNPs contribute to loss of asthma control have not been defined, results from preliminary studies suggest the number of neutrophils that infiltrate into the airway is modulated in individuals with specific P2X₇ genetic variations (31). To facilitate the possible use of P2X₇ status as a novel biomarker for diverse diseases such as asthma, our group has developed a relatively inexpensive and non-invasive blood test to identify patients that possess P2X₇ loss-of-function polymorphisms (68). In this test, <1 ml of whole blood is used in a flow cytometry-based P2X₇ pore formation assay. The blood cells are stimulated with the P2X₇ agonist 2'-3'-O-(4-benzoyl)benzoyl-ATP (BzATP) in the presence of the fluorescent dye YO-PRO[®]-1, which passes through the P2X₇-stimulated pore. The pore is then closed via the addition of MgCl₂, and the uptake of YO-PRO[®]-1 is measured using a flow cytometer. Pore activity below a specified threshold is considered "low" P2X₇ activity, and "low" activity is generally predictive of a P2X₇ loss-of-function polymorphism (66, 68). If P2X₇ antagonists pass clinical trials and/or P2X₇ becomes an established biomarker, the whole blood pore assay could be useful for identifying patients with attenuated P2X₇ function using commonly available clinical equipment.

Anti-P2X₇ Clinical Trials

Because of growing interest in the contribution of P2X₇ to various diseases, numerous pharmaceutical companies have developed P2X₇ antagonists. A few recent reviews offer a comprehensive listing of the P2X₇ antagonist patents that have been documented up to November 2009 (8, 11, 69). In addition to the development of P2X₇-directed compounds, many companies are testing these compounds in clinical trials for the treatment of conditions ranging from rheumatoid arthritis to neurological disorders. AstraZeneca's P2X₇ antagonist AZD-9056 has successfully passed Phase I trials and has recently completed a six month Phase IIb study for the treatment of rheumatoid arthritis (70). AZD-9056 was also examined for the treatment of osteoarthritis, chronic obstructive pulmonary disorder and irritable bowel syndrome, but these studies have since been terminated due to a lack of efficacy. Pfizer's P2X₇ antagonist CE-224535 is currently in Phase II trials for the treatment of rheumatoid arthritis in patients who do not improve after methotrexate treatment (70, 71). As with AZD-9056, CE-224535 was tested as a treatment for osteoarthritis, but it was also terminated due to a lack of efficacy. Evotec is pursuing a "P2X₇ Antagonist Program", and their antagonist EVT 401 has successfully passed Phase I toxicology testing and will be entering Phase II studies for the treatment of rheumatoid arthritis. Additionally, GlaxoSmithKline's P2X₇ antagonist GSK1482160 is currently undergoing toxicology testing for treatment of inflammatory pain, such as that observed in arthritis (70).

Besides the current clinical trials testing P2X₇ antagonists for the treatment of rheumatoid arthritis, several trials have been initiated to investigate the link between neurological disorders and P2X₇ activation. Affectis Pharmaceuticals has synthesized a central nervous system (CNS)-penetrant P2X₇ antagonist, AFC-5128, that is currently being tested as a

potential treatment for neuroinflammatory and neurodegenerative diseases (72). Affectis Pharmaceuticals also recently patented two P2X₇ antagonists for the treatment of depression and bipolar affective disorder (73). Furthermore, it has been reported that AstraZeneca's AZD-9056 may also be used as treatment for mood disorders such as depression (11). P2X₇ antagonists are not the only method being used in clinical trials to target P2X₇ activity, e.g., studies in the Netherlands are currently underway to determine the effect of mechanical stimulation of bone using a Juvent 1000 vibration platform in order to promote ATP release *in vivo* (70). Mechanical stimuli are important for maintaining normal bone structure, and ATP is released from bone cells upon mechanical stress and contributes to bone remodeling by binding to P2 receptors (2, 74). Thus, increasing mechanical stimulation and activating P2 receptors may be a novel therapeutic target for the treatment of skeletal disorders such as osteoporosis. The motivation behind this study is also partially based on the observation that P2X₇ loss-of-function SNPs are associated with fracture risk in a cohort of postmenopausal women (75).

IL-1 β - A Critical Mediator of P2X₇ and Regulator of Inflammatory and Neurologic Functions

One of the most well characterized functions of P2X₇ is its promotion of the processing of IL-1 β (and the related IL-18) into its biologically active form. This process is mediated by the assembly of an inflammasome comprised of caspase-1, Nucleotide-binding and Leucine-Rich Repeat-, and PYD-Containing Protein 3 (NALP3), and Apoptosis-associated Speck-like containing a CARD (ASC) (76). Interestingly, many of the P2X₇ drugs in clinical trials were screened for their ability to attenuate IL-1 β release, a process used to identify compounds with the highest therapeutic potential. IL-1 β is a pro-inflammatory cytokine that plays a role in inflammatory and neurologic disorders such as arthritis, sepsis, Alzheimer's disease, depression and chronic neuropathic pain (77).

The biosynthesis of IL-1 β involves two steps: 1) synthesis of pro-IL-1 β and 2) proteolytic cleavage of pro-IL-1 β by caspase-1 to form the active cytokine. The current consensus is that K⁺ efflux induced by activated P2X₇ stimulates the cleavage of pro-caspase-1 to caspase-1, which consequently can convert IL-1 β to its bioactive form (76). Qu *et al.* proposed that the rapid export of IL-1 β upon stimulation of P2X₇ involves the formation of multivesicular bodies that contain exosomes with bioactive IL-1 β and inflammasome components (78). A role for P2X₇ in IL-1 β processing is well documented in monocytic cells treated with extracellular nucleotides and various Toll-like receptor (TLR) agonists, with LPS being the most extensively examined (79, 80). Macrophages from P2X₇ null mice are deficient in ATP-stimulated IL-1 β release (81), and human monocytic cells primed with LPS release mature IL-1 β via a P2X₇-dependent mechanism (19). In addition to extracellular nucleotides and LPS, other endogenous stimuli have been shown to induce P2X₇-dependent IL-1 β maturation. The human cathelicidin-derived peptide LL37, a potent antibacterial peptide produced by neutrophils and epithelial cells, has been shown to promote the release of mature IL-1 β from LPS-primed monocytes in a P2X₇-dependent manner (82). There is also compelling evidence for a role of the pannexins, which are a family of transmembrane channels, in P2X₇-dependent IL-1 β release (79, 83, 84). In terms of a disease model, LPS-treated monocytes from patients with rheumatoid arthritis produce significantly higher levels of IL-1 β in response to ATP when compared to control patients (85). Thus, P2X₇ plays a critical role in IL-1 β processing and is a potential point of therapeutic intervention for the attenuation of IL-1 β action.

Dysregulation of the processing and release of IL-1 β has been extensively studied in neurological diseases, including Alzheimer's disease. There is accumulating evidence suggesting that the key component in the development of the pathophysiology of

Alzheimer's disease is the inflammatory cycle encompassing IL-1 β and other pro-inflammatory factors. IL-1 β affects many aspects of neurodegeneration, such as the formation of β -amyloid precursor protein, activation of astrocytes, and increased inducible nitric oxide synthase (iNOS) production (77, 86). Multiple studies have revealed that P2X₇ is involved in the processing/release of IL-1 β and the generation of ROS from microglia, macrophages and other monocytic cells (87–90). In mixed glial cell cultures from P2X₇ knockout mice, LPS-induced IL-1 β release, but not TNF α release, is largely suppressed in comparison to wild-type controls (91), supporting the contention that P2X₇ is a critical stimulator of IL-1 β release. In addition, P2X₇ has been reported to regulate the release of IL-1 β and cysteinyl leukotrienes in certain astrocyte cultures (92, 93). Notably, IL-1 β can potentiate its own mRNA expression and P2X₇ expression in microglia and astrocytes, respectively (94, 95). This positive feedback loop continues to drive the inflammatory cycle and results in a reactive glial environment. Ultimately, sustained activation of microglia and astrocytes can lead to an increase in cell death, inflammation, neuronal stress, and cell damage, which positively promote the inflammatory cycle (77, 96).

As discussed above, several animal studies have linked P2X₇ to neuropathic pain, and this connection is attributed to altered IL-1 β production (97). In the study by Chessell *et al.*, P2X₇-deficient mice exhibited a decreased level of systemic IL-1 β following an adjuvant challenge and were protected from inflammatory and neuropathic pain caused by both mechanical and thermal stimuli (45). In animal arthritic models, a P2X antagonist, oATP, has been reported to exert anti-nociceptive effects in rats, and mice lacking P2X₇ display a decrease in the incidence and severity of monoclonal anti-collagen-induced arthritis (36, 81). Collectively, there is considerable data supporting the concept that P2X₇-mediated IL-1 β release plays a role in both chronic inflammatory and neuropathic pain.

P2X₇-Mediated Gene Expression: A Recently Recognized Function

The release of mature IL-1 β is not the only mechanism by which P2X₇ is proposed to regulate inflammation; there is increased appreciation for the idea that P2X₇ activates transcription factors and the synthesis of multiple immuno-modulatory factors in immune cells (12). It is a relatively recently recognized concept that P2X₇ regulates gene transcription. To date, it has been reported that P2X₇ ligands also promote the production of numerous immune mediators, including VEGF, COX-2, IL-2, IL-6, IL-8 and iNOS (20–22, 98–100). In addition, we and others have reported that P2X₇ ligands can stimulate the expression, activation and/or nuclear translocation of several transcription factors, including members of the early growth response (Egr-1, -2 and -3) family, the nuclear factor of activated T cells (NFAT), nuclear factor- κ B (NF- κ B) family members, the cyclic-AMP response element (CRE)-binding protein (CREB), and the AP-1 family members c-Fos, FosB and JunB (20, 30, 100–104). Details about these findings in the context of immune regulation are provided below (also see Fig. 2).

Selected Immune Mediators Regulated by P2X₇

One immune factor that has recently been shown to be regulated at the mRNA level in response to P2X₇ signaling is VEGF, which is a pro-angiogenic factor and a drug target for multiple types of cancer (21, 105). This factor is released from monocytic cells during inflammatory wound repair to promote the formation of new blood vessels and immune cell migration (105, 106). Because both VEGF and high levels of ATP are released from cells upon shear stress (2, 107), we tested the idea that P2X₇ is involved in the release of VEGF. We observed that P2X₇ ligands induce VEGF mRNA synthesis and VEGF protein release from monocytic cells, and that a P2X₇ antagonist attenuates ligand-stimulated VEGF secretion (21). In support of these data, Wei *et al.* showed that the P2X₇ agonist BzATP induces VEGF mRNA expression in rat C6 glioma cells (99). Thus, it is possible that

extracellular nucleotides regulate the resolution of inflammation by stimulating this pro-angiogenic factor.

IL-8 is an important chemokine that attracts immune cells such as neutrophils to sites of infection (108), and it has been demonstrated that P2X₇ ligands induce the expression of this factor. Specifically, Wei *et al.* reported that BzATP stimulates IL-8 mRNA production in the C6 glioma cell line, and Idzko *et al.* showed that ATP and BzATP stimulate IL-8 release from primary human blood eosinophils (99, 109). In addition, Schneider *et al.* reported that human peripheral blood mononuclear cells release IL-8 upon ATP stimulation (110). Because ATP reportedly stimulates the recruitment of neutrophils to sites of tissue injury, and P2X₇ pore function reportedly correlates with the change in nasal lavage neutrophil counts during a cold, it is interesting to speculate that these processes are partially mediated by P2X₇ ligand-induced IL-8 expression (31, 111).

IL-2 is a well characterized cytokine that is involved in the development of memory and regulatory T cells (112), and it is considered an attractive therapeutic target for cancer and autoimmune diseases. Recombinant IL-2 has been used to treat melanoma and renal cell carcinoma, and several immuno-suppressive drugs function by inhibiting the IL-2 signaling cascade (113–115). In terms of a connection between extracellular nucleotides and IL-2 generation, several groups have reported that P2X₇ ligands can induce the expression of IL-2. For example, Budagian *et al.* showed that ATP induces IL-2 mRNA expression in Jurkat T cells, and Yip *et al.* validated this finding in the same cell line (100, 116). Also, Woehrl *et al.* reported that siRNA against P2X₇ attenuates ATP-induced IL-2 expression in Jurkat cells (117). These results support the idea that extracellular nucleotides may regulate adaptive immunity.

Another protein involved in mediating many inflammatory and vascular responses is iNOS (118), which is an enzyme that catalyzes the synthesis of NO from L-arginine. During an inflammatory response, iNOS promotes the production of high NO concentrations that are capable of stimulating microbial killing (118). In this regard, we have reported that BzATP enhances LPS-induced iNOS expression in macrophages (89, 102), and Gendron *et al.* presented evidence that BzATP enhances IFN γ -stimulated iNOS expression and NO production in BV-2 microglial cells (119). Thus, the production of iNOS by extracellular nucleotides may be one mechanism by which these ligands stimulate the killing of intracellular pathogens such as *M. tuberculosis* (120).

The pro-inflammatory enzyme COX-2 catalyzes a key step in the conversion of arachidonic acid into biologically active prostaglandins, and it is the target of several anti-inflammatory drugs (121). We have found that P2X₇ ligands induce the expression of COX-2 in an osteoblast cell line and in human peripheral blood mononuclear cells (15, 20). Interestingly, COX-2 is a proposed regulator of bone turnover, and Panupinthu *et al.* reported that two COX-2 inhibitors reduce BzATP-stimulated mineralization and gene expression in calvarial cultures (122, 123). In accordance with the observation that COX-2 expression can be regulated by P2X₇ stimulation, the production of prostaglandins, particularly prostaglandin E₂ (PGE₂), by ATP has also been attributed to P2X₇ stimulation. Specifically, extracellular ATP-induced PGE₂ release from osteoblasts appears to be P2X₇-dependent (40). Furthermore, fluid shear stress, which is known to induce the release of ATP, significantly increases PGE₂ release in calvarial osteoblasts from P2X₇ wild-type mice but has no significant effect on PGE₂ release in osteoblasts isolated from P2X₇ knockout mice (40). Osteoblast stimulation has been linked to PGE₂ release, which is important for osteoclast differentiation and bone resorption, as well as the pathogenesis of rheumatoid arthritis (124, 125). Therefore, P2X₇ regulation of COX-2 expression appears important in the mediation of both inflammation and bone formation/remodelling.

Transcriptional Regulators and P2X₇ Action

It is probable that additional immuno-modulatory gene targets of P2X₇ signaling will soon be identified and that the mechanisms by which these targets are induced will be deciphered. In this regard, the activation of transcription factors (Egr, NFAT, NF- κ B, CREB, AP-1) are likely to be key transcriptional regulators mediating the actions of P2X₇ ligands, and these factors are discussed below (also see Fig. 2).

Egr

Egr-1, Egr-2 and Egr-3 are immediate early genes that play important roles in immunity, and the expression of these zinc finger transcription factors is induced by multiple mitogenic signals in various cell types. Egr-1 is involved in the activation of B and T cells while Egr-2 and Egr-3 are thought to promote anergy in T cells (126). Interestingly, it has been recently reported that P2X₇ ligands induce the expression of these factors (103, 104). Specifically, Stefano *et al.* used HEK293 cells that express P2X₇ to demonstrate that BzATP can promote the transcriptional up-regulation of Egr-1 via the action of ERK1/2 and the transcription factor Elk-1, supporting the notion that P2X₇ activation influences gene transcription (104). In addition, Friedle *et al.* showed that BzATP induces Egr-1, Egr-2, and Egr-3 expression in N9 microglia and primary microglia cells (103). In these studies, it was also noted that the introduction of Egr RNAi into N9 microglia attenuates BzATP-stimulated IL-6 production, as well as the production of TNF- α induced by co-stimulation with LPS and BzATP (103). Furthermore, it has been reported that Egr-1 potentiates VEGF expression in lung cancer cells (127), suggesting that the P2X₇-dependent VEGF release we observed with human monocytes may arise via Egr-1 activation.

NFAT

The NFAT transcription factor family (NFAT1–5) regulates the expression of many immune mediators. NFAT is activated by a rise in cytoplasmic Ca⁺⁺ levels achieved by the release of Ca⁺⁺ stores from the endoplasmic reticulum and the opening of Ca⁺⁺-release-activated Ca⁺⁺ channels. This rise in intracellular Ca⁺⁺ activates calmodulin, which activates the serine/threonine phosphatase calcineurin. Calcineurin then dephosphorylates NFAT and exposes a nuclear import sequence, allowing the transcription factor to translocate into the nucleus and promote gene transcription (128, 129). Several groups have provided evidence that NFAT is regulated by P2X₇; thus, activation of this transcription factor family by nucleotides may be a key mechanism by which the expression of immune mediators is regulated by P2X₇. Yip *et al.* showed that P2X₇ RNAi in Jurkat T cells attenuates NFAT activity and IL-2 transcription induced by a T cell activating factor (100). Adinolfi *et al.* demonstrated that the P2X inhibitor oxidized ATP (oATP) decreases NFAT1 nuclear expression in HEK293 cells exogenously expressing P2X₇, and Kataoka *et al.* showed ATP activates NFAT1 in MG-5 microglial cells (101, 130). Additionally, Ferrari *et al.* reported that ATP and BzATP activate NFAT in N9 microglial cells (131).

NF- κ B

NF- κ B is a widely distributed transcription factor that modulates the induction of numerous immuno-modulatory genes, including IL-6, IL-8, TNF- α , iNOS and COX-2 (132, 133). This factor exists in an inactive state in the cytoplasm through its association with inhibitor of κ B (I κ B). Upon phosphorylation of I κ B by I κ B kinase (IKK), I κ B becomes marked for degradation by the proteasome and NF- κ B translocates into the nucleus where it can promote gene transcription (132, 133). We and others have reported that P2X₇ agonists stimulate NF- κ B translocation and DNA binding activity in multiple cell types. For example, Ferrari *et al.* showed ATP stimulates NF- κ B binding to DNA in the mouse microglial cell lines N9 and N13, and that this binding was inhibited by antioxidant

treatment, whereas Budagian *et al.* reported that ATP stimulates NF- κ B binding to DNA in Jurkat T cells (116, 134). Similarly, Korcok *et al.* demonstrated BzATP induces the nuclear translocation of NF- κ B in mouse osteoclasts but not in osteoclasts from P2X₇ knockout mice (135), and we showed that BzATP induces NF- κ B DNA binding in RAW 264.7 macrophages and in human peripheral blood mononuclear cells (15, 98, 102).

CREB

The leucine zipper transcription factor CREB functions to control many cell processes, including glucose homeostasis, cell survival, and inflammatory mediator production. Phosphorylation of CREB on serine-133 stimulates co-activation by CREB-binding protein (CBP) or p300, allowing CREB to bind to conserved CREB response elements (CREs; 5'-TGACGTCA-3') in the promoters of cAMP-responsive genes. Several immuno-modulatory genes including IL-2, IL-6, IL-10 and TNF- α contain CRE sites in their promoters (136). We have reported that P2X₇ activation can stimulate CREB function in monocytic cells (30). We found that stimulation with the P2X₇ agonist BzATP results in phosphorylation of CREB at serine-133 and that this signaling event is dependent upon ERK1/2 and Ca⁺⁺ (30). Ligands for P2X₇ can lead to CREB activation in human peripheral blood mononuclear cells, the human monocytic cell line THP-1, the murine macrophage cell line RAW 264.7, and in HEK293 cells that exogenously express P2X₇. We also showed that RAW 264.7 cells that express a loss-of-function P2X₇ mutation, i.e., RAW S342F cells, do not exhibit CREB phosphorylation in response to BzATP but do exhibit activation of CREB in response to LPS or anisomycin (an activator of p38 MAPK) (30). In addition, Potucek *et al.* reported that BzATP activates CREB in BV-2 microglia cells (137). In further support of a role for P2X₇ in the transcriptional regulation of gene expression, we have shown that inhibition of CREB activation using dominant negative CREB constructs significantly attenuates the expression of the AP-1 genes c-Fos and FosB (20, 30).

AP-1

The AP-1 transcription factors are stimulated by many factors, including UV light, growth factors and oxidative stress, and they regulate the transcription of genes important for metastasis, invasion, differentiation, hypoxia, angiogenesis, proliferation, and apoptosis (138). This family of transcription factors includes c-Jun, JunB, JunD, Fra-1, Fra-2, c-Fos, FosB and Δ FosB, and various dimers of these factors bind to AP-1 sites in gene promoters (the AP-1 consensus sequence is TGAC/GTCA). AP-1 family members contain a basic leucine zipper region that is important for dimerization with another AP-1 protein and for DNA binding. Transcriptionally active AP-1 proteins include a Jun protein and a Fos family member (138). We and other groups have reported that P2X₇ ligands can induce the expression and activation of AP-1 transcription factors (20, 116, 139). We found that P2X₇ ligands modestly induce c-Fos and JunB expression, but can robustly promote the rapid (*i.e.*, hrs) induction of FosB and Δ FosB in macrophages and osteoblasts, and we also observed that these agonists stimulate the binding of FosB proteins to DNA (20). FosB and Δ FosB are two of the lesser-studied AP-1 family members, but increasing evidence supports a role for these transcription factors in inflammation, bone turnover, and neuronal function. The biology of FosB and Δ FosB and the potential significance of our observation in the context of immune, osteogenic, and neuronal regulation will be discussed below.

FosB and Δ FosB

The AP-1 transcription factors FosB and Δ FosB are poorly understood, but the further investigation of their contributions to neurobiology and immunology appears warranted. These factors are most well known for their roles in adaptive changes in the brain in response to stimulation with drugs of abuse, and Δ FosB is hypothesized to be a “molecular switch” in the development of drug addiction (140). Overall, much less is known about

FosB and Δ FosB in comparison to the other AP-1 family members, but it is known that FosB and Δ FosB possess a PEST domain (a sequence rich in proline, glutamate, serine, threonine), a basic region, and a leucine zipper. The leucine zipper is the major site of interaction between FosB and its AP-1 binding partner Jun. Δ FosB is a truncated splice variant of FosB that lacks 101 amino acids from the C-terminus of FosB and a portion of the transactivation domain found in full-length FosB. Δ FosB mRNA is spliced from full-length FosB by the excision of 140 nucleotides of “intronic” sequence in the open reading frame in FosB. This splicing event induces a frameshift that creates a stop codon in Δ FosB (141). Δ FosB is quite stable because it lacks two destabilizing elements (a proteasome-dependent and a proteasome-independent degron) that are present in full-length FosB; thus, its half-life is longer than FosB in many systems (142).

There is considerable interest in Δ FosB in psychiatric research because Δ FosB accumulates in region-specific areas of the brain after chronic stimuli with an array of drugs or following stress, and its expression persists for weeks to months. It is thought that Δ FosB regulates long-term adaptive changes in the brain by altering transcriptional profiles and by controlling natural and drug-induced reward (143). Previous reports have shown that Δ FosB can be induced by cocaine, amphetamine, morphine, nicotine, alcohol, antidepressants, antipsychotic drugs, chronic exercise, sucrose, and sexual reward. Furthermore, Δ FosB remains expressed in the brain for several weeks or more after cessation of the stimulus (143–149). Nestler *et al* and McClung *et al* offer comprehensive reviews on the long-term persistence of Δ FosB in the brain (140, 143), and numerous animal model studies support the idea that Δ FosB regulates behavioral plasticity and is critical for normal psychiatric behavior. One of the first FosB knockout studies demonstrated that FosB knockout mice exhibit normal cognitive and sensory functions but they do not nurture their offspring; thus, their pups require surrogate parents (150). As for regulating behavioral plasticity, it was discovered early that FosB knockout mice display exaggerated responses to initial cocaine exposure but fail to become sensitized after repeated exposure (151). Subsequent studies have demonstrated the importance of Δ FosB in regulating reward by both natural stimuli and drugs of abuse. For example, mice that over-express Δ FosB in the nucleus accumbens exhibit increased sensitivity to drugs of abuse and drug-seeking behavior (152, 153).

Several reports have provided evidence that FosB and Δ FosB may possess broader functions, including the capacity to modulate the inflammatory response and bone formation. In terms of inflammation, it has been reported that FosB becomes transcriptionally active in mouse lungs after mechanical ventilation (154), and Δ FosB accumulates and persists in lumbar segments of rat spinal cords after injection of λ -carrageenan type IV, a molecule used to induce inflammatory pain (155). In addition, studies have shown oxidative stress induced by H_2O_2 promotes the expression of FosB (156). Furthermore, FosB expression is higher in inflammatory breast cancer tissue in comparison to non-inflammatory tissue controls (157). As for bone formation, FosB knockout mice do not display any skeletal abnormalities (158), whereas Δ FosB transgenic mice exhibit increased progressive osteosclerosis, or augmented bone formation, that appears to be osteoblast-dependent (159). The mechanisms by which Δ FosB regulates bone turnover are not well defined, but it has been reported that FosB/ Δ FosB is required for mechanical stress-induced production of IL-11 in osteoblasts (160). As noted above, mechanical stress stimulates ATP release from bone cells and contributes to bone remodeling (2). As for IL-11, this factor regulates normal bone remodeling through the regulation of both osteoclasts and osteoblasts, and it has been demonstrated that IL-11 promotes osteoblast differentiation *in vitro* (161). It is possible that ATP induces the expression of FosB and Δ FosB via the action of P2X₇, and that these events result in the transcription of osteogenic genes such as IL-11.

Examination of the Evidence for an ATP/P2X₇/FosB Pathway

We have reported the existence of a previously unrecognized ATP/P2X₇/FosB pathway in monocytic and osteoblast cells (20) and propose that this signaling network contributes to the transcriptional regulation of multiple immuno-modulatory factors. We have found that transient stimulation with P2X₇ ligands (BzATP, ATP) induces the expression of FosB and Δ FosB in human peripheral blood mononuclear cells, RAW 264.7 macrophages, mouse bone marrow-derived macrophages, the preosteoblast cell line MC3T3-E1, and HEK293 cells that stably express P2X₇ (20 and data not shown). In addition to showing P2X₇ ligands induce the expression of FosB and Δ FosB, we also used electrophoretic mobility shift assays (EMSA) to demonstrate that BzATP activates FosB proteins in RAW 264.7 and MC3T3-E1 cells (20). In these studies, cells were treated with agonist for 5 min, the media was replaced, and the cells were harvested 0.5–3 h later. The short-term (5 min) treatment with agonist may be more representative of an *in vivo* microenvironment where bursts of high levels of nucleotides are achieved upon tissue damage or platelet degranulation, and these high concentrations are normalized after nucleotide breakdown by ectonucleotidases. Strikingly, low levels of BzATP (5–30 μ M) that do not stimulate most P2X₇-mediated events, such as ion channel activation and pore formation (7), can induce the expression of FosB and Δ FosB (ref. (20) and data not shown). These data support the idea that FosB and Δ FosB are two of the most sensitive known mediators of P2X₇ action. We reported that both FosB and Δ FosB become expressed 1 h after BzATP treatment, and that the FosB proteins persist for at least 3 h. Recent work from our laboratory has shown that FosB and Δ FosB are substantially degraded in RAW 264.7 cells at a time 8 h post-BzATP treatment (data not shown), which is consistent with results in rat adrenal pheochromocytoma (PC12) cells showing FosB and Δ FosB are degraded within 8 h following treatment with 20% serum (142). Therefore, it is unlikely that nucleotides stimulate long-term persistence of FosB and Δ FosB in macrophages as observed in models of drug addiction where Δ FosB persists for days to weeks in the brain (143).

To determine the biological significance of the robust induction of FosB and Δ FosB in response to nucleotides, we tested the idea that FosB and Δ FosB are required for P2X₇-mediated induction of pro-inflammatory proteins. To test our hypothesis, we used RNAi to knock down FosB expression in MC3T3-E1 osteoblasts, treated the cells with BzATP for 5 min, replaced the media, and prepared cell lysates after 4 h to assess COX-2 expression using immunoblotting (20). We observed that P2X₇ ligand-mediated COX-2 induction in MC3T3-E1 cells is dependent upon FosB proteins (20), revealing that at least one key endpoint of P2X₇ signaling is linked to FosB expression. Accordingly, it is of interest to ascertain whether the ATP/P2X₇/FosB signaling axis functions in monocytic cells to regulate the expression of other immuno-modulatory factors.

It is known that the P2X₇ agonists BzATP and ATP can activate other P2X family members (7); therefore, to verify that P2X₇ mediates the induction of FosB and Δ FosB, we performed several control experiments and utilized a recently developed P2X₇ inhibitor (20). We observed that BzATP-mediated FosB and Δ FosB induction is substantially attenuated in RAW 264.7 cells that express the loss-of-function P2X₇ S342F mutant, and BzATP is unable to induce FosB-DNA binding activity (as indicated by an EMSA) in the P2X₇-defective cell line. Furthermore, it was noted that the P2X₇ antagonist A438079 reduces BzATP-stimulated FosB and Δ FosB induction in RAW 264.7 macrophages (20). Thus, we conclude that P2X₇ stimulates FosB and Δ FosB expression in response to nucleotides, but it is currently unknown whether other P2X family members may also induce the expression of these transcription factors.

Future Studies

The nucleotide receptor P2X₇ is a key mediator of the inflammatory response, and increasing evidence implicates that it also performs important functions in bone and neuronal regulation. Numerous studies support the idea that P2X₇ is an attractive therapeutic target for inflammatory conditions; thus, P2X₇ inhibitors are in clinical trials for rheumatoid arthritis, and several groups are attempting to develop additional P2X₇ antagonists (162–166). Furthermore, recent reports support the hypothesis that P2X₇ induces the transcription of immuno-modulatory genes, and that this action is likely a key mechanism by which P2X₇ mediates inflammatory, osteogenic and neuronal functions.

Our recent analyses of P2X₇-stimulated transcriptional responses have revealed that P2X₇ activates CREB and the intriguing, yet poorly understood, AP-1 transcription factors FosB and ΔFosB. In addition, we have found that FosB/ΔFosB is involved in P2X₇ ligand-stimulated COX-2 induction in osteoblast cells, supporting the idea that this signaling axis controls the induction of inflammatory mediators. The finding that extremely low doses of P2X₇ ligands can promote the potent induction of FosB/ΔFosB in macrophages and osteoblasts suggests that this system is responsible for some of the most essential, and perhaps unrecognized, actions of P2X₇. Little is known regarding the role of FosB and ΔFosB in the inflammatory response when compared to the amount of data establishing their role in behavioral plasticity, and thus defining the significance of this transcriptional regulatory system in immune function is of great interest. Similarly, it is unknown whether P2X₇ polymorphisms contribute to altered inflammatory mediator production via dysregulated FosB/ΔFosB production/action, and this topic is also an area of active investigation. Many disorders with chronic inflammation such as asthma and rheumatoid arthritis are attributed, at least in part, to genetic factors, but the genes and polymorphisms that promote these disorders have not been clearly identified. Because FosB and ΔFosB are stimulated by low concentrations of nucleotides, it is possible that P2X₇ is constitutively being activated to induce these transcription factors and regulate basal production of immuno-modulatory proteins.

As briefly discussed above, ATP, P2X₇, and FosB proteins are thought to regulate bone formation. Two major cell types that regulate bone formation are osteoclasts (resorb mineralized bone) and osteoblasts (form bone), and these cell types coordinately function together to ensure normal bone turnover. Although FosB knockout mice do not display detectable skeletal deformities (158), mice that over-express ΔFosB appear to exhibit osteoblast-dependent osteosclerosis (159), whereas P2X₇ knockout mice display excessive trabecular bone resorption and deficient periosteal bone formation (39). The role of P2X₇ in osteoclast formation and regulation is complex; therefore, additional studies are required to determine whether the skeletal deformities observed in P2X₇ knockout mice are attributed to altered osteoclast function (2). However, it has been reported that P2X₇ ligands stimulate osteoblast differentiation and mineralization; thus, altered osteoblast function may be a mechanism by which P2X₇ knockout animals display skeletal defects. Because we have shown P2X₇ ligands induce FosB and ΔFosB in the osteoblast cell line MC3T3-E1, it is possible that the ATP/P2X₇/FosB pathway is activated in this cell type to regulate bone formation and normal bone turnover. The identification of transcriptional targets of the ATP/P2X₇/FosB pathway in osteoblasts will facilitate the identification of the mechanisms by which extracellular nucleotides regulate this cell type.

In terms of neuronal function, there is accumulating evidence that both P2X₇ and FosB proteins play critical roles in a psychiatric context. P2X₇ function has been attributed to depression, and a P2X₇ non-synonymous SNP has been correlated with affective mood disorders (52, 62, 63, 65). Furthermore, the roles of FosB and ΔFosB in drug addiction and

reward from various stimuli (natural and drugs of abuse) are well established. In a depression study using P2X₇ knockout mice, it was demonstrated that animals deficient in P2X₇ have less depressive symptoms than wild-type controls, suggesting that P2X₇ can contribute to depressive behavior (52). If the depressive behavior mediated by P2X₇ is linked to the induction of ΔFosB, one would expect that animals over-expressing ΔFosB would also exhibit symptoms of depression. However, Berton *et al.* reported the opposite effect in mice over-expressing ΔFosB in the ventrolateral periaqueductal gray; the authors found that mice with the strongest induction of ΔFosB in this region were the most resilient to inescapable stress, which is an animal model for affective disorder (167). Thus, P2X₇ likely promotes depressive behaviors using a non-FosB-dependent mechanism, e.g., P2X₇ knockout mice may have coped better with inescapable stress because of a deficiency in IL-1β processing (52). Nonetheless, it is reasonable to speculate that extracellular nucleotides and FosB/ΔFosB play a role in drug addiction. It is not known whether P2X₇ is expressed in reward pathways in the brain or whether P2X₇ induces FosB and ΔFosB expression in neuronal cells. If extracellular nucleotides do induce ΔFosB in region-specific areas of the brain important for drug tolerance and reward, it would be fascinating to test the hypothesis that polymorphisms in P2X₇ can contribute to individual patterns of drug abuse.

In sum, the field of P2X₇ biology is rapidly growing, yet much is left to be determined concerning the mechanisms by which this receptor regulates immunity, bone formation, and neurologic function. Recent evidence supporting a function for extracellular nucleotides in the regulation of gene transcription offers new insight into the mechanisms by which multiple physiological responses are controlled by P2X₇.

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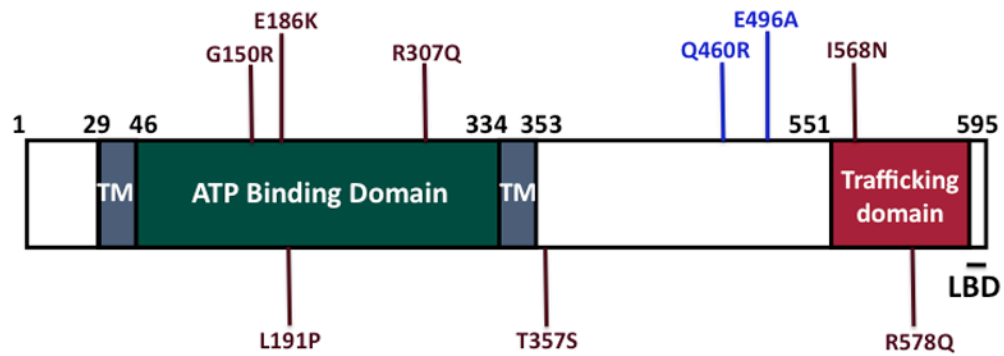


Fig. 1. Architecture of Human P2X₇

Human P2X₇ possesses a predicted extracellular ATP binding domain, two transmembrane domains (TM), an intracellular trafficking domain, and a lipid binding domain (LBD). In addition, numerous P2X₇ SNPs have been identified, including several with reduced function. Non-synonymous SNPs that exhibit reduced activity are represented in brown, and SNPs that have been correlated with human disease are shown in blue

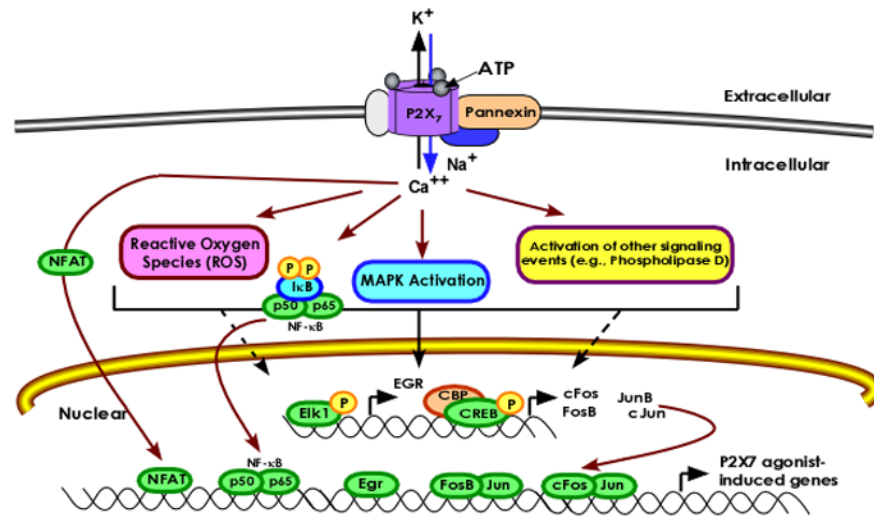


Fig. 2. Summary Model of the Proposed Mechanisms of P2X₇-mediated Control of Transcriptional Regulators

Stimulation of the ionotropic nucleotide receptor P2X₇ with extracellular ATP induces the efflux of K⁺ and the influx of Ca⁺⁺ and Na⁺. Following prolonged receptor stimulation, there is the formation of a nonselective pore that has been reported to involve the pannexins. Upon ligand binding, P2X₇ can initiate multiple signaling events, including the production of reaction oxygen species, the activation of multiple MAP kinase (MAPK) cascades, and the expression/activation of numerous transcription factors (shown in green). The role of the various P2X₇-initiated signaling cascades in the activation of these transcription factors is still poorly understood, but many reports support a central role for intracellular Ca⁺⁺ fluxes and MAPK signaling cascades. For example, Ca⁺⁺ fluxes appear linked to the nuclear translocation of the NFAT, whereas reactive oxygen species production and Ca⁺⁺ fluxes have been linked to NF-κB nuclear translocation, and MAPK-dependent events appear essential for the activation of the CREB and Egr transcription factors. In addition, the transcriptional regulation and expression of only a small number of P2X₇-dependent genes has been elucidated to date, including the P2X₇-mediated expression of several Egr and AP-1 (cFos, FosB, JunB) transcription factors. Pannexins: family of transmembrane channels; important in P2X₇-mediated IL-1β processing and release. I-κB: NF-κB regulatory protein that is degraded after phosphorylation, allowing NF-κB translocation into the nucleus. p50/p65: NF-κB subunits proposed to be P2X₇ agonist-induced. Elk-1 (Ets LiKe gene-1): Ets transcription factor of the ternary complex factor subfamily that binds to the serum response element in the promoter of genes; linked to the expression of Egr1 after P2X₇ activation. CBP (CREB-binding protein): required for CREB-mediated gene expression; P2X₇ agonists induce CREB/CBP complex formation. P: phospho-group; regulates the activation of various proteins. Jun (JunB, cJun): members of the AP-1 family of transcription factors; JunB and cJun expression is upregulated after P2X₇ stimulation

Table 1
P2X₇ Non-synonymous SNPs

Several P2X₇ polymorphisms are listed including their location, reported functionality, and links with disease

SNP	Characteristics
V76A	Reduced channel and pore activities (27)
R117W	Reduced channel and pore activities (27)
G150R	Reduced channel and pore activities (27)
H155Y	Increased channel and pore activities (27, 60)
A176V	Within the extracellular domain
E186K	Next to critical N-linked glycosylation site (13); reduced channel and pore activities (27)
L191P	Near critical N-linked glycosylation site (13); reduced channel and pore activities (27)
R270H	No change in function (66)
R270C	Within the extracellular domain
R276H	Within the extracellular domain
R307Q	Reduced channel and pore activities; within the ATP binding pocket; expresses on the cell surface (66, 168)
L320P	Within the extracellular domain
A348T	Increased channel and pore activities; correlated with <i>T. gondii</i> infection (27, 67)
T357S	Reduced channel and pore activities (27, 66)
I367T	Located in the intracellular domain near the second transmembrane domain
P430R	Within the intracellular domain
A433V	Within the intracellular domain
Q460R	Functionality is unclear; correlated with major depressive and bipolar disorders in some studies (62, 63, 65)
E496A	Reduced pore activity but may possess normal channel function; correlated with papillary thyroid cancer, tuberculosis (27, 33, 54, 58–60)
H521Q	Within the intracellular domain
V522I	Normal channel and pore activities (27)
R544Q	Our preliminary data suggests no change in function
I568N	Reduced channel and pore activity; within the C-terminal trafficking domain; does not express on the cell surface (27, 169)
R578Q	Previous studies and our preliminary data suggest it has reduced pore and signaling functions; within the C-terminal trafficking domain (12, 26)