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SYMPOSIUM REVIEW

TRPM2: a multifunctional ion channel for calcium signalling

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The transient potential receptor melastatin-2 (TRPM2) channel has emerged as an important Ca^{2+} signalling mechanism in a variety of cells, contributing to cellular functions that include cytokine production, insulin release, cell motility and cell death. Its ability to respond to reactive oxygen species has made TRPM2 a potential therapeutic target for chronic inflammation, neurodegenerative diseases, and oxidative stress-related pathologies. TRPM2 is a non-selective, calcium (Ca^{2+})-permeable cation channel of the melastatin-related transient receptor potential (TRPM) ion channel subfamily. It is activated by intracellular adenosine diphosphate ribose (ADPR) through a diphosphoribose hydrolase domain in its C-terminus and regulated through a variety of factors, including synergistic facilitation by $[Ca^{2+}]_i$, cyclic ADPR, H_2O_2, NAADP, and negative feedback regulation by AMP and permeating protons (pH). In addition to its role mediating Ca^{2+} influx into the cells, TRPM2 can also function as a lysosomal Ca^{2+} release channel, contributing to cell death. The physiological and pathophysiological context of ROS-mediated events makes TRPM2 a promising target for the development of therapeutic tools of inflammatory and degenerative diseases.

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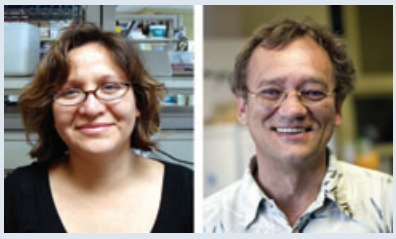
Introduction

Transient receptor potential (TRP) proteins represent a large superfamily of six-transmembrane (6TM) monovalent and divalent cation-permeable ion channels that are homologues of the *Drosophila melanogaster* TRP protein, a Ca^{2+}-permeable channel that is essential for phototransduction (Ramsey *et al.* 2006; Nilius, 2007;



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Venkatachalam & Montell, 2007). The history of TRP proteins began with the discovery of a mutant strain of *Drosophila melanogaster* that had an abnormal response to prolonged illumination and therefore was visually impaired (Cosens & Manning, 1969). This led to the isolation of the first TRP protein in 1989 (Montell & Rubin, 1989) and subsequent identification of mammalian TRP-like proteins (Ramsey *et al.* 2006; Nilius, 2007; Venkatachalam & Montell, 2007).

Currently, the mammalian TRP superfamily is composed of 28 members which are grouped into six subfamilies based on their amino acid sequence homology: canonical or classic (C), vanilloid (V), melastatin (M), polycystin (P), mucolipin (ML) and ankyrin (A). Typically, the TRP protein structure is characterized by six-transmembrane domains, a pore region situated between the fifth and sixth transmembrane domains, with N- and C-termini oriented toward the cytoplasm (Ramsey *et al.* 2006; Nilius, 2007; Venkatachalam & Montell, 2007; Gaudet, 2008, 2009; Latorre *et al.* 2009; Song & Yuan, 2010). To form a channel, TRP proteins typically assemble into homo-tetramers, although in some cases hetero-tetramers have been demonstrated to form functional channels (Goel *et al.* 2002; Hofmann *et al.* 2002; Li *et al.* 2006).

Even within the major subfamilies, TRP channels exhibit a remarkable diversity in their mechanisms of activation (thermally activated, receptor activated and ligand activated), selectivity and permeability to cations (Na^+, Ca^{2+}, Mg^{2+}, K^+) (Ramsey *et al.* 2006; Nilius, 2007; Venkatachalam & Montell, 2007). Activation of TRP channels in the plasma membrane mediates the flux of Ca^{2+} and/or Na^+ into the cell, thereby raising intracellular concentration of these ions and depolarizing the cell (Ramsey *et al.* 2006; Nilius, 2007; Venkatachalam & Montell, 2007). Additionally, intracellular TRP channels may function as Ca^{2+} release channels and modulate the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (Lange *et al.* 2009; Dong *et al.* 2010; Gees *et al.* 2010). TRP channels are widely expressed in different tissues and cell types from worms, fruit flies, zebrafish, mice and humans (Venkatachalam & Montell, 2007), including excitable and non-excitable cells, and they can be found in the plasma membrane and organelles, demonstrating the diversity of their biological roles (Ramsey *et al.* 2006; Nilius, 2007; Venkatachalam & Montell, 2007). They are involved in sensory functions such as taste transduction and temperature sensation (Talavera *et al.* 2008), in homeostatic functions like Ca^{2+} and Mg^{2+} reabsorption and osmoregulation, or cellular functions like cell motility and muscle contraction (Venkatachalam & Montell, 2007; Gees *et al.* 2010).

The TRP-melastatin subfamily (TRPM), named for the tumour suppressor melastatin (TRPM1), contains eight mammalian members: TRPM1–TRPM8, which are divided into four homologue pairs: TRPM1/TRPM3, TRPM2/TRPM8, TRPM4/TRPM5 and TRPM6/TRPM7 (Fleig & Penner, 2004*a*,*b*). The N-terminus of the TRPM subfamily members is characterized by four stretches of residues, designated as the TRPM homology domain or MHD (Perraud *et al.* 2001). Additionally, TRPM proteins present a TRP domain within their C-termini (LPPPFI) near the transmembrane segments (Venkatachalam & Montell, 2007). Several TRPM channels (TRPM1–6, and TRPM8) are expressed as multiple variants or isoforms, either as a full-length protein or a short variant, contributing to their biological complexity (Vazquez & Valverde, 2006; Venkatachalam & Montell, 2007). It has been suggested that these splice variants may play a role modulating the function, ion selectivity and localization of these channels. However, the significance of most of them has not yet been determined. In this review we focus on the TRPM2 channel and discuss its molecular and biophysical properties, as well as the physiological context in which it functions.

TRPM2 structure

TRPM2 (previously known as LTRPC2 or TRPC7) is a multifunctional Ca^{2+} permeable, non-selective cation channel with a unique C-terminal adenosine diphosphate ribose (ADPR) pyrophosphatase domain (Nudix-like domain or NUDT9 homology domain) (Perraud *et al.* 2001; Sano *et al.* 2001; Fleig & Penner, 2004*a*,*b*). Like TRPM6/7, TRPM2 is known as a 'chanzyme' because of its dual function of ion channel and C-terminal enzyme domain. The human TRPM2 gene is located in chromosome 21q22.3, consists of 32 exons and spans approximately 90 kb (Nagamine *et al.* 1998). An additional 5′-exon contained within a CpG island has been identified in the human TRPM2 gene (Uemura *et al.* 2005). On the other hand, the mouse TRPM2 gene contains 34 exons and spans about 61 kb (Uemura *et al.* 2005). The human TRPM2 transcript is ∼6.5 kb and encodes a protein of 1503 amino acids (1507 in mouse and rat) with a predicted molecular mass of ∼170 kDa (Nagamine *et al.* 1998; Hara *et al.* 2002; Jiang *et al.* 2010) (Fig. 1). The TRPM2 protein structure comprises six transmembrane segments (S1–S6), flanked by the intracellular N- and C-termini, with the pore-forming loop domain located between S5 and S6 (Nagamine *et al.* 1998; Perraud *et al.* 2001; Sano *et al.* 2001). Additionally, the TRPM2 N-terminus has four homologous domains and a calmodulin (CaM) binding IQ-like motif, which plays a role in modulating channel activation (Perraud *et al.* 2001; Sano *et al.* 2001; McHugh *et al.* 2003; Fleig & Penner, 2004*a*,*b*; Tong *et al.* 2006) (Fig. 1). The significance of the MHD domains in TRPM2 function or expression remains to be established. On the other hand, the C-terminus contains a TRP box and a

coil–coil domain (Jiang, 2007), which has been suggested to be critical for the homo-tetrameric assembly of TRPM2 (Fig. 1).

ADPR is considered the primary gating molecule of TRPM2 (Perraud *et al.* 2001; Sano *et al.* 2001; Fleig & Penner, 2004*a*, *b*). It binds to the Nudix-like domain in the C-terminus of TRPM2 with high specificity and is subsequently hydrolysed (K_m 100 μM, V_{max} 100 nmol μg^{-1} min^{-1}) to ribose 5-phosphate and AMP (Bessman *et al.* 1996; Perraud *et al.* 2001, 2003*a*,*b*) (Fig. 1). Although the physiological role of TRPM2 enzymatic activity has not been explored in detail, it is generally assumed that the enzymatic activity may serve to provide negative feedback inhibition for TRPM2 activity, since AMP antagonizes ADPR-mediated gating of TRPM2 (Kolisek *et al.* 2005; Beck *et al.* 2006; Lange *et al.* 2008). Upon binding of ADPR, TRPM2 channels will open and allow the permeation of sodium (Na^+), potassium (K^+) and Ca^{2+} into the cell with a relative permeability of P_{Ca}:P_{Na} ~0.3–0.9 (Perraud *et al.* 2001; Sano *et al.* 2001; Venkatachalam & Montell, 2007) (Fig. 1). TRPM2 currents are characterized by a linear current–voltage (*I–V*) relationship with a reversal potential of ~0 mV (Perraud *et al.* 2001; Sano *et al.* 2001). The single channel conductance is ~60 pS with unusually long open times in the range of several seconds (Perraud *et al.* 2001).

TRPM2 gating by ADPR is subject to remarkable modulatory mechanisms, both inhibitory and facilitatory. Negative regulators include AMP (Kolisek *et al.* 2005; Beck *et al.* 2006; Lange *et al.* 2008) and protons (Du *et al.* 2009*b*; Starkus *et al.* 2010; Yang *et al.* 2010), whereas facilitation is observed with Ca^{2+} (McHugh *et al.* 2003; Starkus *et al.* 2007; Csanady & Torocsik, 2009), hydrogen peroxide (H_2O_2) (Hara *et al.* 2002; Kolisek *et al.* 2005), cyclic ADPR (cADPR) (Kolisek *et al.* 2005; Lange *et al.* 2008), and nicotinic acid adenine dinucleotide phosphate (NAADP) (Beck *et al.* 2006; Lange *et al.* 2008). Some of these modulatory effects appear to be directly mediated at the channel protein, whereas others are indirect, involving cytosolic components yet to be identified (Toth & Csanady, 2010).

TRPM2 is expressed at its highest in the brain but is also detected in other tissues such as bone marrow, spleen, heart, liver and lung, and in different cell types like pancreatic β-cells (Ishii *et al.* 2006*b*; Togashi *et al.* 2006; Lange *et al.* 2009; Uchida *et al.* 2010), endothelial cells (Hecquet *et al.* 2008; Hecquet & Malik, 2009; Hecquet *et al.* 2010), microglia (Kraft *et al.* 2004), neurons (Hill *et al.* 2006; Olah *et al.* 2009), cardiomyocytes (Yang *et al.* 2006), and immune cells (neutrophils, megakaryocytes, monocytes/macrophages) (Heiner *et al.* 2003; Carter *et al.* 2006; Lange *et al.* 2008; Yamamoto *et al.* 2008).

Although it was originally described as a plasma membrane channel, TRPM2 has recently been found to also function as a lysosomal Ca^{2+} release channel in pancreatic β-cells (Lange *et al.* 2009). Intracellular localization, albeit not in lysosomes, has also been reported for other TRP channels, including TRPV1 (Morenilla-Palao *et al.* 2004), TRPC5 (Bezzerides *et al.* 2004), TRPC3 (Singh *et al.* 2004), TRPM8 (Thebault *et al.* 2005), TRPML1–3 (Piper & Luzio, 2004; Cheng *et al.* 2010; Dong *et al.* 2010), and TRPM7 (Oancea *et al.* 2006). The factors that would determine the cellular localization TRPM2 and various other TRP channels remain to be defined, as well as whether the cellular localization serves a particular cellular function.

TRPM2 activation and modulation

ADPR is the most efficient TRPM2 activator, with variable EC_{50} values of 1–90 μM depending on the cell type investigated (Perraud *et al.* 2001; Sano *et al.* 2001; Inamura

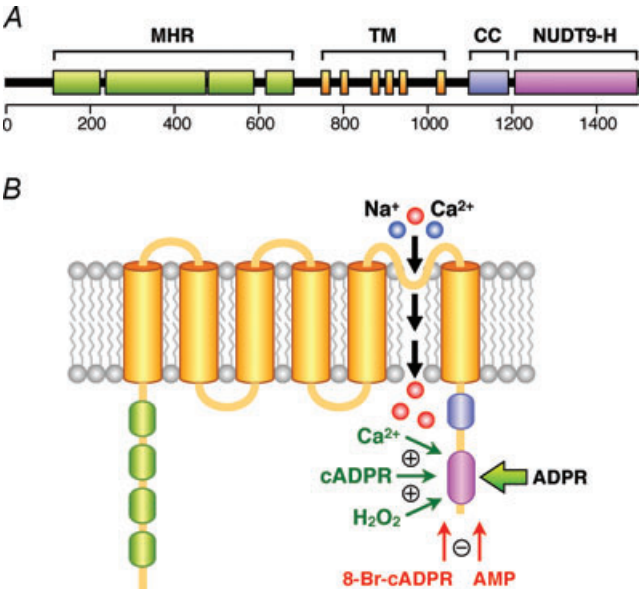

Figure 1. TRPM2 protein structure and transmembrane topology
A, TRPM2 protein structure. Human TRPM2 is a protein of ~170 kDa composed of 1503 amino acids (1507 in mouse and rat). The channel's N-terminal has four homologous regions (MHR) of unknown function and a calmodulin (CaM) binding IQ-like motif, followed by six transmembrane segments (TM: S1–S6). The TRPM2 pore-forming loop domain locates between S5 and S6. The TRPM2 C-terminus contains a TRP box and a coil–coil domain (CC), and a C-terminal adenosine diphosphate ribose (ADPR) pyrophosphatase domain (Nudix-like domain or NUDT9 homology domain, NUDT9-H). *B*, TRPM2 transmembrane topology. The TRPM2 N- and C-termini face the cytosol. Cytosolic ADPR binds to the TRPM2 NUDT9-H region and gates the channel, allowing calcium (Ca^{2+}) and sodium (Na^+) influx. ADPR is hydrolysed to ribose 5-phosphate and adenosine monophosphate (AMP) by TRPM2 NUDT9-H enzymatic activity. TRPM2 gating by ADPR is facilitated by hydrogen (H_2O_2), cyclic ADPR (cADPR) and Ca^{2+}. AMP acts as a negative regulator of TRPM2 gating by ADPR and 8Br-cADPR inhibits cADPR- and H_2O_2-mediated effects.

et al. 2003; Beck *et al.* 2006; Gasser *et al.* 2006; Starkus *et al.* 2007; Lange *et al.* 2008). The variability in EC_{50} values may arise from the modulatory mechanisms expressed in a given cell type. The main cellular pathways that generate free ADPR are the hydrolysis of NAD^+ and/or cADPR by glycohydrolases, including the ectoenzymes CD38 and CD157 as well as the mitochondrial NADase (Lund *et al.* 1995, 1998; Lund, 2006; Malavasi *et al.* 2006). A further source of ADPR is provided by the combined action of poly(ADPR) polymerases (PARPs, PARP enzymes) and poly(ADPR) glycohydrolases (PARG enzymes), which indirectly generate ADPR via formation and hydrolysis of poly-ADPR when hyperactivated in response to DNA damage (Caiafa *et al.* 2009; Esposito & Cuzzocrea, 2009; Fauzee *et al.* 2010).

Other nucleotides with the ability to activate TRPM2 channels, although less effectively, are cADPR ($EC_{50} = 0.7$ mM) (Kolisek *et al.* 2005; Lange *et al.* 2008), and NAADP ($EC_{50} = 0.73$ mM) (Beck *et al.* 2006; Lange *et al.* 2008). Even though activation of TRPM2 by high concentrations of NAD^+ ($EC_{50} = 1$–1.8 mM) has been observed (Sano *et al.* 2001; Hara *et al.* 2002; Naziroglu & Luckhoff, 2008), its status as a direct agonist for TRPM2 remains to be established more thoroughly, since at least in some studies, contaminations with ADPR or metabolism of NAD^+ may account for the observed TRPM2 activation by high concentrations of NAD^+ (Beck *et al.* 2006; Grubisha *et al.* 2006). The concentrations of cADPR and NAADP required to activate TRPM2 are high compared with the physiological concentration of these nucleotides; however, these nucleotides can synergize with ADPR and increase TRPM2 sensitivity at much lower doses. Whether they bind directly to the Nudix domain or to different cooperative sites, or are converted to ADPR is not clearly understood. Nevertheless, abundant evidence suggests that TRPM2 activation is linked to pathways that involve the generation of these nucleotides (Fig. 2).

CD38, a multifunctional ectoenzyme widely expressed in haematopoietic cells and non-haematopoietic tissues, uses NAD^+ as a substrate to catalyse the production of ADPR, cADPR and NAADP (Lund *et al.* 1995, 1998). CD38 knock-out (KO) neutrophils stimulated with the bacterial peptide formyl-methionyl-leucyl-phenylalanine (fMLP) present a reduced Ca^{2+} response when compared to wild-type cells (Partida-Sanchez *et al.* 2003). Similarly, fMLP-treated TRPM2 KO neutrophils have defects in Ca^{2+} influx (Yamamoto *et al.* 2008). Additionally, the fMLP-induced Ca^{2+} entry can be inhibited when neutrophils are treated with 8Br-ADPR or 8Br-cADPR (Partida-Sanchez *et al.* 2004; Partida-Sanchez *et al.* 2007). Interestingly, TRPM2 channels are expressed in the plasma membrane of neutrophils, suggesting a signalling pathway involving CD38–ADPR–TRPM2. Although ADPR is the main product of CD38 and evidence points to TRPM2 as the mediator of the Ca^{2+} entry, there are still open questions such as how the extracellular ADPR generated by CD38 crosses the plasma membrane and acts on the cytosolic Nudix domain of TRPM2 channels.

A novel acetyl-ADP ribose product, *O*-acetyl-ADP ribose (OAADPR), which is derived from the histone/protein deacetylase reaction mediated by sirtuins, induces TRPM2 currents by direct binding to the Nudix domain with a K_d of $\sim$100 μM (Grubisha *et al.* 2006; Tong & Denu, 2010). SIRT2 and SIRT3, mammalian sirtuins, have been suggested to generate the OAADPR that leads to TRPM2-dependent cell death induced by puromycin, since specific RNAi knockdown in TRPM2-expressing cells protects these cells from cell death (Grubisha *et al.* 2006). This suggests that, OAADPR may also function as a physiological regulator of TRPM2.

Extracellular Ca^{2+} and intracellular Ca^{2+} have also a critical role in the full activation of TRPM2 channels (McHugh *et al.* 2003; Starkus *et al.* 2007; Csanady & Torocsik, 2009). Intracellular Ca^{2+} facilitates TRPM2 activation by enhancing the channel sensitivity to ADPR. ADPR alone in the complete absence of Ca^{2+} cannot induce cation currents and a minimum of 30 nM internal Ca^{2+} is required to enable TRPM2 currents. However, this facilitation is not mimicked by Mg^{2+}, Ba^{2+}, or Zn^{2+} (Starkus *et al.* 2007). On the other hand, 200 μM external Ca^{2+} is as efficient as 1 mM Ca^{2+} in TRPM2 activation (Starkus *et al.* 2007). It has also been suggested that Ca^{2+} can gate the channel directly in a dose-dependent manner, with an EC_{50} of 17 μM, or 0.5 μM in the presence of 10 μM ADPR (McHugh *et al.* 2003; Starkus *et al.* 2007; Du *et al.* 2009*a*). Although the mechanism is still not clear it is thought to result from conformational changes due to $[Ca^{2+}]_i$-dependent tethering of CaM with TRPM2 IQ-like motif or other intracellular sites (Du *et al.* 2009*a*). However, other investigations did not observe Ca^{2+}-induced activation (McHugh *et al.* 2003; Starkus *et al.* 2007; Csanady & Torocsik, 2009) and it is possible that Ca^{2+} at high concentrations could lead to ADPR production or ADPR release from mitochondria.

TRPM2 channels can also be activated by H_2O_2 (Hara *et al.* 2002; Kolisek *et al.* 2005) and other agents that produce oxygen and nitrogen species, although the mechanism of action remains unclear. It appears that the gating mechanism relies primarily on its ability to release ADPR from mitochondria (Ayub & Hallett, 2004). In addition, it has been shown that H_2O_2-mediated TRPM2 currents can be suppressed by reducing the ADPR concentration within the mitochondria (Perraud *et al.* 2005). Contrasting this hypothesis, a TRPM2 variant lacking the Nudix domain still responds to H_2O_2 (Zhang *et al.* 2003). Another source of ADPR under oxidative stress is the nucleus. ADPR generation in the nucleus involves the activation of PARP-1/PARG pathway by DNA damage. PARP-1 binds to damaged-DNA and catalyses the cleavage

of NAD^+ to nicotinamide and ADPR (Caiafa *et al.* 2009; Esposito & Cuzzocrea, 2009; Fauzee *et al.* 2010). ADPR is then polymerized onto various nuclear proteins, activating DNA repair mechanisms. Free ADPR is generated following the degradation of ADPR polymers by PARG. The suppression of the oxidative stress TRPM2-mediated Ca^{2+} response by PARP inhibitors suggests that the H_2O_2 effect involves the production of free ADPR (Fonfria *et al.* 2004). Additionally, PARP-deficient DT40 cells, which express TRPM2, exhibit no oxidative stress Ca^{2+} responses (Buelow *et al.* 2008). Interestingly, H_2O_2-mediated Ca^{2+} influx via TRPM2 is negatively affected when experiments are performed at room temperature and recovers at 37°C (Hermosura *et al.* 2008; Wilkinson *et al.* 2008). Moreover, it has been shown that temperatures >35°C may activate TRPM2 channels or facilitate their activation by ADPR or cADPR in rat insulinoma RIN-5F cells (Togashi *et al.* 2006). However, the mechanisms involved in the temperature-dependent regulation of TRPM2 remain to be explored in more detail.

TRPM2 currents are also regulated by cellular acidification (Du *et al.* 2009*b*; Starkus *et al.* 2010; Yang *et al.* 2010). TRPM2 currents are completely suppressed when exposing cells to external or internal pH of 5–6 (Du *et al.* 2009*b*; Starkus *et al.* 2010). Although both extracellular and intracellular mechanisms have been proposed to account for the proton-mediated regulation of TRPM2, the simplest interpretation that is compatible with the data reported so far would suggest that protons compete with Na^+ and Ca^{2+} for channel permeation, and channel closure results from a competitive antagonism of protons at an intracellular Ca^{2+} binding site (Csanady, 2010; Starkus *et al.* 2010). The physiological relevance of this mechanism of TRPM2 regulation is unknown. However, it may be important both pathologically during tissue acidification caused by, for example, injury, inflammation, tumours, or ischaemia (Stekelenburg *et al.* 2008; Holzer, 2009; Stock & Schwab, 2009), as well as physiologically in highly acidic stores like lysosomes, where TRPM2 is also expressed and acts as a Ca^{2+} release channel.

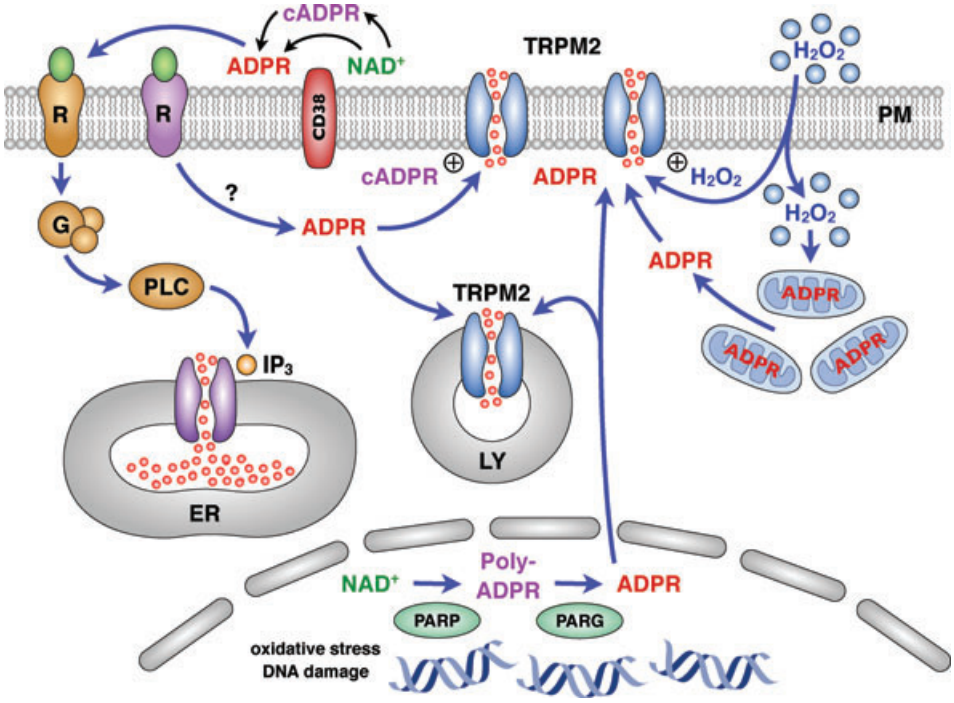

Figure 2. Signalling mechanisms for TRPM2 activation
NAD^+ and reactive oxygen species (ROS), including H_2O_2, accumulate during inflammation and tissue damage. External NAD^+ may be converted to ADPR, cADPR and NAADP by the ectoenzymes CD38 and CD157. Extracellular ADPR may then bind to plasma membrane receptors (e.g. P2Y receptors) and increases $[Ca^{2+}]_i$ through Ca^{2+} release from stores via G-proteins and phospholipase C (PLC) activation with subsequent IP_3 production. H_2O_2 may also cross the plasma membrane and mobilize ADPR from mitochondria (both H_2O_2 and cADPR can synergize with ADPR to activate TRPM2). ADPR is also generated from poly-ADPR during ROS-induced DNA damage through activation of the PARP-1/PARG pathway. Free cytosolic ADPR will act on the NUDT9-H of lysosomal and plasma membrane TRPM2 channels, enabling Ca^{2+} influx across the plasma membrane and/or release of lysosomal Ca^{2+}, raising the Ca^{2+} concentration in the cytosol. Ca^{2+} overload can trigger programmed cell death (apoptosis) and possibly necrosis. Finally, extracellular signals that remain to be identified could potentially induce the production of intracellular free ADPR, which may then gate TRPM2 channels in the lysosome and/or plasma membrane and regulate receptor-mediated signalling.

The nucleotide adenosine monophosphate (AMP) can also antagonize TRPM2 gating by ADPR with an IC_{50} of 70 μM in HEK cells overexpressing TRPM2 channels (Kolisek *et al.* 2005) and 10 μM is sufficient to suppress endogenous channels in neutrophils (Lange *et al.* 2008). The antagonism is thought to result from a competition for the Nudix domain, which itself generates AMP from ADPR through enzymatic pyrophosphatase activity. This could potentially represent a physiological autoregulatory negative feedback mechanism of TRPM2 activity. It is additionally possible that AMP regulation of TRPM2 is less direct (Toth & Csanady, 2010) by involving, for example, AMP-dependent kinase modulation of TRPM2.

TRPM2 currents can also be inhibited pharmacologically by 8Br-cADPR (Kolisek *et al.* 2005; Beck *et al.* 2006), 8Br-ADPR (Partida-Sanchez *et al.* 2007), flufenamic acid (50–1000 μM) (Hill *et al.* 2004*a*), imidazole antifungal agents (clotrimazole and econazole; both at 3–30 μM) (Hill *et al.* 2004*b*), *N*-(*p*-amylcinnamoyl) (Kraft *et al.* 2006), anthranilic acid (ACA; 1.7 μM) and 2-aminoethoxydephenyl borate (2-APB; 1.2 μM) (Kraft *et al.* 2006; Togashi *et al.* 2008). However, most of these molecules are not very potent, nor do they exhibit high TRPM2 specificity.

While cytosolic ADPR appears to be the primary activator of TRPM2, the nucleotide also has extracellular effects. External application of ADPR increases $[Ca^{2+}]_i$ through Ca^{2+} release from stores in TRPM2 expressing rat pancreatic β-cell line RIN-5F, an effect that is blocked by inhibiting phospholipase C (PLC) and IP_3 (Ishii *et al.* 2006*a*). This observation led our group to investigate ADPR-induced Ca^{2+} pathways in pancreatic β-cells (Lange *et al.* 2009). Application of 100 μM ADPR to intact wild-type HEK293 cells, which do not express native TRPM2 channels, produced a transient Ca^{2+} signal independently of extracellular Ca^{2+} or the expression of TRPM2 in the plasma membrane of these cells. This response was blocked by suramin, suggesting that ADPR activates P2Y receptors. Both pathways, P2Y and TRPM2 dependent, were confirmed in the pancreatic β-cell line INS-1, where ADPR additionally activates adenosine receptors. Thus, ADPR appears to represent a multifunctional messenger that can activate membrane receptors extracellularly and also serve as an intracellular gating mechanism for TRPM2 residing in the plasma membrane and intracellular organelles.

Alternative splicing isoforms

The first TRPM2 variants were identified in the monocytic cell line HL-60 and in neutrophil granulocytes as two short proteins, the TRPM2 ΔN which is missing 20 residues (K538–Q557) in the N-terminus and the TRPM2 ΔC lacking 34 residues (T1292–L1325) in the C-terminus (Wehage *et al.* 2002). TRPM2 ΔC responds to H_2O_2 but not to ADPR, supporting the hypothesis of direct activation of TRPM2 by H_2O_2 (Wehage *et al.* 2002). In contrast, TRPM2 ΔN fails to respond to H_2O_2 (Wehage *et al.* 2002). However, a later study failed to confirm the C terminus as a locus for direct H_2O_2 activation (Kuhn & Luckhoff, 2004).

A third variant, TRPM2-S (short), contains only the N-terminus and the first two transmembrane segments and is generated by an additional stop codon between exons 16 and 17. This variant has been found in bone marrow, brain and pulmonary arteries and aorta (Zhang *et al.* 2003; Vazquez & Valverde, 2006; Yang *et al.* 2006; Hecquet & Malik, 2009) and may act as a dominant negative inhibitor of TRPM2 activity (Zhang *et al.* 2003).

A further isoform has been detected in human striatum, but not in mouse (Uemura *et al.* 2005). This variant, named striatum short protein (SSF)-TRPM2, contains the transmembrane domains and the C-terminal region, including the Nudix domain, but lacks the N-terminal 214 amino acid residues and retains H_2O_2-induced Ca^{2+} influx activity (Uemura *et al.* 2005). Consistent with this, SSF-TRPM2 localizes in the plasma membrane when expressed in HEK293 cells.

Computational analysis of altered methylation patterns of DNA regions with tumoral interest, has also detected two interesting TRPM2 transcripts in melanoma, the melanoma-enriched antisense TRPM2 transcript, TRPM2-AS, and a tumour-enriched TRPM2 transcript, TRPM2-TE (Orfanelli *et al.* 2008). Both transcripts seem to be up-regulated in malignant melanoma with little or no detectable levels in normal melanocytes (Orfanelli *et al.* 2008). Their transcription site appears to be in the intron 24 of the TRPM2 gene. In addition, there appears to exist a TRPM2-TE variant lacking part of exon 26 and the entire exon 27 (like the TRPM2 ΔC splice variant), resulting in the deletion of 34 amino acids from the Nudix domain but still containing the elements for the enzymatic activity (Orfanelli *et al.* 2008). The mechanisms of expression and functions of these transcripts remain to be characterized in more detail (Vazquez & Valverde, 2006). Nevertheless, the plethora of TRPM2 transcripts indicates that they might represent adaptive mechanisms for the cellular regulation of TRPM2 channels in different tissues, cell types and (patho)physiological circumstances.

TRPM2 and pathologies

TRPM2 has been associated with diseases such as bipolar disorder and type II diabetes. However, little is known about the TRPM2-dependent pathways that are affected in these pathologies. TRPM2 can mobilize Ca^{2+} from both extracellular and intracellular compartments and as such its biological significance is related to cellular

functions that are regulated by $[Ca^{2+}]_i$. Since TRPM2 is most abundantly expressed in the brain, it is not surprising that TRPM2 has also been associated with CNS pathologies. Patients with bipolar disorders type I present high basal $[Ca^{2+}]_i$, and the chromosome region 21q22.3 harbours genes that confer susceptibility to this pathology, including TRPM2 (Xu *et al.* 2006, 2009). Although TRPM2 variants with a single amino substitution (e.g. Asp543Glu) have been detected in patients with bipolar disorder, the relevance of these variants in the pathogenesis of the disease remains to be elucidated. TRPM2 has also been linked to amyotrophic lateral sclerosis and parkinsonism–dementia (Hermosura & Garruto, 2007). Here, a TRPM2 mutation (P1018L) results in channels that inactivate more rapidly than wild-type channels, resulting in reduced Ca^{2+} entry. Again, the cellular and functional context of TRPM2 in these pathologies remains to be demonstrated.

In addition to the CNS, TRPM2 has also been identified in pancreatic β-cells Qian *et al.* 2002; Ishii *et al.* 2006*a*,*b*; Togashi *et al.* 2006; Bari *et al.* 2009; Lange *et al.* 2009). Moreover, TRPM2 activity has been linked to insulin secretion and alloxan- and H_2O_2-mediated apoptosis of insulin-secreting cells, suggesting a role of TRPM2 in diabetes (Herson & Ashford, 1997; Herson *et al.* 1999; Togashi *et al.* 2006; Uchida *et al.* 2010). A recent study using the TRPM2 KO mouse model revealed involvement of this channel in insulin secretion in mouse pancreatic β-cells (Uchida *et al.* 2010). In this study, basal blood glucose levels were higher in TRPM2-KO mice than in WT mice, while plasma insulin levels were similar. In isolated β-cells, TRPM2-KO cells produced smaller Ca^{2+} signals in response to high concentrations of glucose and incretin hormone than WT cells, resulting in reduced insulin secretion from pancreatic islets of TRPM2-KO mice. These results indicate that TRPM2 is involved in insulin secretion stimulated by glucose. In contrast, a clinical study of a patient population with type 2 diabetes mellitus found no association of the genetic TRPM2 variants rs2838553, rs2838554, rs4818917, rs1619968, rs1785452, rs2238725, rs2010779, rs9979491 and rs1573477 with this chronic disease (Romero *et al.* 2010). However, the variants rs2838553, rs2838554 and rs4818917 showed negative association with a homeostatic model assessment of β-cell function, which determines insulin resistance and β-cell function, hinting at the possibility that TRPM2 activity might regulate β-cell function. Further studies examining other variants are necessary to establish a role of TRPM2 in diabetes.

TRPM2 has also been identified in numerous cell types of the immune system, including neutrophils, monocytes, macrophages and lymphocytes. The ability of H_2O_2 to activate TRPM2 channels has attracted interest to this channel as a potential mechanism for pathogenic processes that are characterized by an increased oxidative microenvironment, including carcinogenesis, inflammation, ischaemia–reperfusion injury, neurodegenerative disorders, diabetes and others. Endogenously, H_2O_2 is generated through oxidative phosphorylation in mitochondria (Roede & Jones, 2010). Exogenously, H_2O_2 generation is induced in concert with other reactive oxygen species (ROS), such as superperoxides (O_2^-) and hydroxyl radicals ($OH^{\cdot}$), as a result of exposure to a wide variety of external factors including certain drugs, pollutants, heavy metals, heat, UV or visible light, and other forms of ionizing radiation (Cheeseman & Slater, 1993). Uncontrolled generation of ROS causes significant damage to a wide range of biological molecules such as DNA, lipids, proteins, carbohydrates or any nearby molecule causing a cascade of chain reactions resulting in cellular damage and disease (Akyol *et al.* 2002). H_2O_2 is an oxidizing agent, and although it is not especially reactive, it is a source of OH^- in the presence of reactive transition metal ions, which is an extremely reactive oxidizing radical that affects most biomolecules (Naziroglu, 2007).

H_2O_2 also functions as an antimicrobial agent in immune cells and as a signal-transduction molecule (Droge, 2002). Importantly, H_2O_2 activates TRPM2 and in cells that express TRPM2 at high levels, this can induce cell death by sustained increases in $[Ca^{2+}]_i$ (Hara *et al.* 2002). The mechanism by which H_2O_2 activates TRPM2 channels is not entirely resolved. The slow kinetics of TRPM2 activation by external application of H_2O_2 suggests that H_2O_2 needs to cross the plasma membrane and generate ADPR or other factors to modulate TRPM2. Mutations in the Nudix domain or treatment with PARP inhibitors prevents or strongly impairs TRPM2 activation by H_2O_2 (Fonfria *et al.* 2004). $[Ca^{2+}]_i$ increases following external H_2O_2 application are also lost in PARP-deficient DT40 cells (Buelow *et al.* 2008).

The significance of the TRPM2 channel in processes that depend on oxidative stress was recently confirmed for dextran sulfate sodium-induced colitis in mice, a model for human inflammatory bowel disease (Yamamoto *et al.* 2008). TRPM2 KO mice were protected from tissue damage due to reduced production of CXCL2 chemokines by monocytes, resulting in diminished recruitment of neutrophils to the site of inflammation. Although neutrophils also express TRPM2, the chemotactic response to CXCL2 was not perturbed in TRPM2-deficient mice. Therefore, impaired neutrophil infiltration to the site of injury in TRPM2KO mice was a consequence of a defective production of CXCL2 in monocytes or macrophages (Yamamoto *et al.* 2008). The impaired chemokine production in cells lacking TRPM2 was linked to a defect in TRPM2-mediated Ca^{2+} influx, which normally proceeds via activation of the Ca^{2+}-dependent kinase Pyk2 and activation of the Erk/NFkB pathway (Yamamoto *et al.* 2008, 2010).

The suppression of H_2O_2-mediated Ca^{2+} signalling and CXCL8 (the human equivalent of CXCL2) production through the Erk pathway was also confirmed in the human monocytic cell line U-937 (Yamamoto *et al.* 2008).

Conclusions

Since the discovery of TRPM2 a decade ago, there have been many important findings that have advanced the understanding of its protein structure and the mechanisms that regulate the activity of this channel. There have also been important advances in the understanding at the gene expression level and most recently its cellular localization and function. This channel has revealed many fascinating features that make it one of the most versatile and intriguing ion channels. Future work will focus on mechanisms that regulate TRPM2 abundance in the various cellular compartments it can be expressed in and what factors determine intracellular *versus* plasma membrane localization. Given the highly integrative nature of this channel, it is likely that additional cellular regulatory factors will be discovered. Further investigations will focus on signalling pathways upstream of TRPM2, so as to determine the cellular mechanisms that mobilize ADPR under physiological and pathological conditions. Finally, it will be interesting to learn to what extent and in what role TRPM2 participates in pathophysiological conditions, both in the CNS and in peripheral tissues. Hopefully, the discovery of pharmacological tools will aid in characterizing TRPM2, with the prospect of using some of these tools therapeutically.

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