*J Physiol* 594.15 (2016) pp 4151–4169



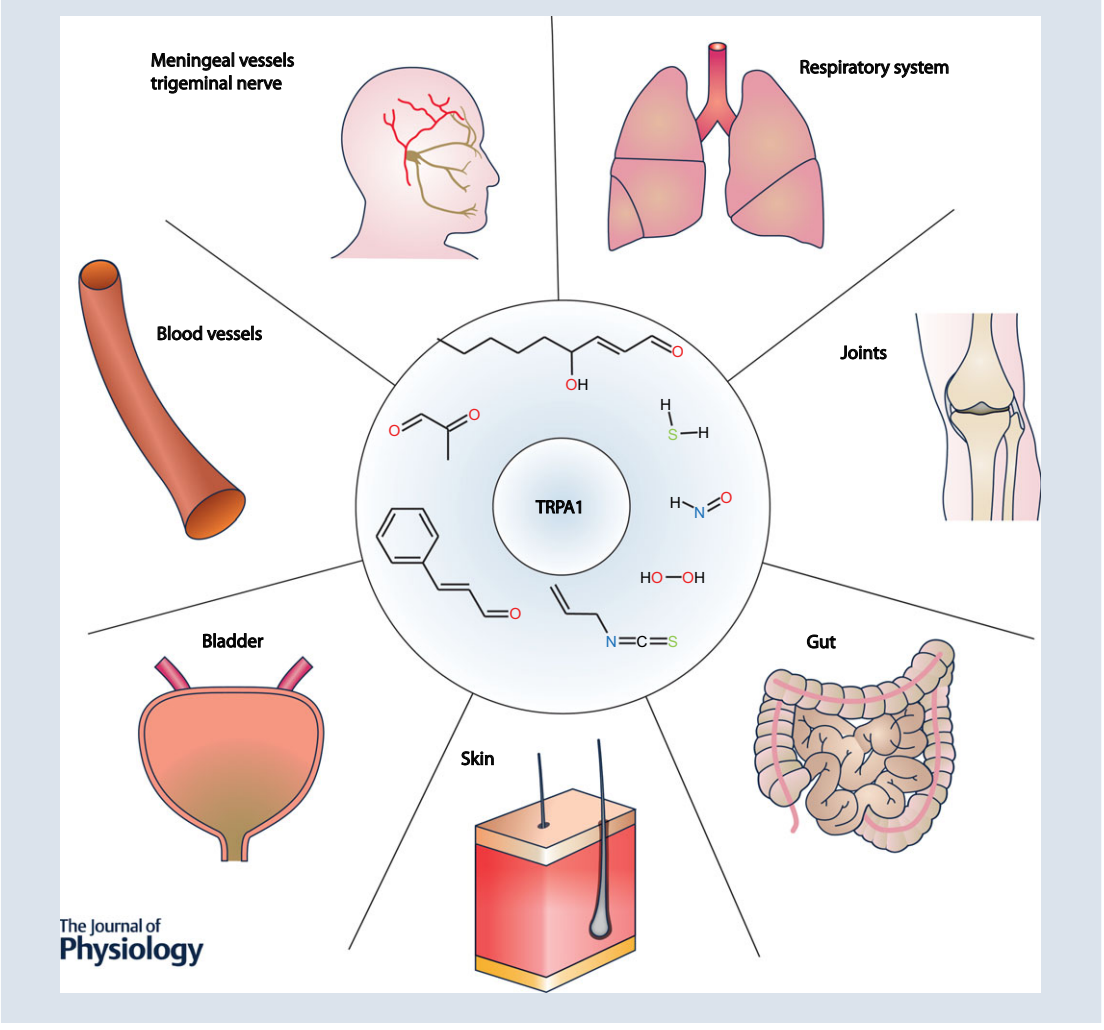
JPn Neuroscience
The Journal of Physiology

SYMPOSIUM REVIEW

# TRPA1 channels: molecular sentinels of cellular stress and tissue damage

Félix Viana

*Instituto de Neurociencias de Alicante, Universidad Miguel Hernández-CSIC, Alicante, Spain*




**Félix Viana** studied Medicine at the University of Santiago de Compostela (Spain). He received his PhD in Physiology and Biophysics at the University of Washington (Seattle), studying developmental aspects of mammalian motoneuron excitability under the mentorship of Albert J. Berger. He gained further insight into the physiological roles played by ion channels in neurons and non-excitable cells during postdoctoral training with Bertil Hille (Seattle) and Bernd Nilius (Leuven). After returning to Spain and establishing his laboratory at the Institute of Neurosciences in Alicante, he developed an interest in the molecular and cellular mechanisms of somatosensory transduction, working closely with his colleague Carlos Belmonte. Currently he is Tenured Investigator of the Spanish Council for Scientific Research (CSIC) and Co-Director of the Sensory Transduction and Nociception Group.

This review was presented at the symposium "Non-selective cationic channels in chemical and physical stress?", which took place at Physiology 2015 in Cardiff, UK, 6–8 July 2015.



DOI: 10.1113/JP270935

**Abstract** TRPA1 is a non-selective cation channel expressed in mammalian peripheral pain receptors, with a major role in chemonociception. TRPA1 has also been implicated in noxious cold and mechanical pain sensation. TRPA1 has an ancient origin and plays important functions in lower organisms, including thermotaxis, mechanotransduction and modulation of lifespan. Here we highlight the role of TRPA1 as a multipurpose sensor of harmful signals, including toxic bacterial products and UV light, and as a sensor of stress and tissue damage. Sensing roles span beyond the peripheral nervous system to include major barrier tissues: gut, skin and lung. Tissue injury, environmental irritants and microbial pathogens are danger signals that can threaten the health of organisms. These signals lead to the coordinated activation of the nociceptive and the innate immune system to provide a homeostatic response trying to re-establish physiological conditions including tissue repair. Activation of TRPA1 participates in protective neuroimmune interactions at multiple levels, sensing ROS and bacterial products and triggering the release of neuropeptides. However, an exaggerated response to danger signals is maladaptive and can lead to the development of chronic inflammatory conditions.

(Received 23 December 2015; accepted after revision 31 March 2016; first published online 15 April 2016)
**Corresponding author** F. Viana: Instituto de Neurociencias de Alicante, Universidad Miguel Hernández-CSIC, Avda. S. Ramón y Cajal s.n., San Juan de Alicante 03550, Spain. Email: felix.viana@umh.es

**Abstract figure legend** TRPA1 is emerging as an important therapeutic target to treat different pathologies, including pain, asthma and chronic itch. However, the broad expression profile of TRPA1 and the fine balance between physiological and maladaptive responses of TRPA1 suggest potential complications. Therefore, a better understanding of TRPA1 function is essential before we can realize the hope of targeting it safely and effectively to treat disease.

**Abbreviations** AITC, allyl isothiocyanate; ARD, ankyrin repeat domain; CFA, complete Freund's adjuvant; CGRP, calcitonin gene-related peptide; cryo-EM, electron cryomicroscopy; DAMP, damage-associated molecular pattern; DRG, dorsal root ganglion; GPCR, G protein-coupled receptor; HNO, nitroxyl anion; LPS, lipopolysaccharide; MSU, monosodium urate; NO, nitric oxide; NOX, NADPH oxidase; PAMP, pathogen-associated molecular pattern; PRR, pattern recognition receptor; P-CTX-1, Pacific Ocean ciguatoxin 1; PUFA, polyunsaturated fatty acid; RNS, reactive nitrogen species; ROS, reactive oxygen species; TLR, Toll-like receptor; TRP, transient receptor potential; UV, ultraviolet.

## Introduction

Nociceptors are specialized primary sensory neurons essential for the perception of pain (reviewed by Woolf & Ma, 2007), an unpleasant feeling or sensation that is a vital component of the body's defence system. Activation of nociceptors sends warning signals to the brain, protecting animals from potential tissue injury (reviewed by Basbaum *et al.* 2009). Together with the innate immune system, nociceptors represent a first line of defence against different (i.e. physical or chemical) potentially damaging environmental agents. Nociceptor activation by harmful stimuli triggers reactions (e.g. motor reflexes, autonomic responses, cough) that minimize exposure to the irritating agent or their consequences. Nociceptors have been classified based on various criteria: conduction velocity, axon diameter or expression of biochemical and molecular markers. Functionally, nociceptors can be classified based on their responses to noxious cold, noxious heat and high threshold mechanical stimuli as well as their response to a variety of chemical substances (e.g. protons, capsaicin, ATP). The molecular characterization of nociceptors and their transduction mechanisms have progressed rapidly (reviewed by Woolf & Ma, 2007; Thakur *et al.* 2014). An important achievement was the identification of TRPV1 as the molecular receptor for capsaicin, the pungent ingredient of chili peppers (Caterina *et al.* 1997). This discovery triggered the identification and characterization of other transient receptor potential (TRP) channels involved in nociception (Dhaka *et al.* 2006). This review is focused on transient receptor potential cation channel subfamily A member 1 (TRPA1) (Story *et al.* 2003), a polymodal channel linked to thermosensation (warm and cold), mechanotransduction and chemosensitivity to irritant compounds (reviewed by Bautista *et al.* 2013; Zygmunt & Högestätt, 2014). Here we highlight the role of TRPA1 as a polymodal sensor of biological signals, including toxic bacterial products, and as a sensor for stress and tissue damage. These alert functions are not limited to the peripheral nervous system but include protective roles in many barrier tissues including the lung and the gut. The final part includes a discussion of new findings concerning TRPA1 function in various diseases.

## A brief primer of TRPA1 structure, expression and function

TRPA1 is a calcium permeable non-selective cation channel belonging to the large TRP family of ion channels (Julius, 2013). Early studies on the expression pattern of

TRPA1 and its sensitivity to physical and chemical stimuli suggested a role in acute thermal nociception (i.e. noxious cold) (Story *et al.* 2003), hair cell mechanotransduction (Corey *et al.* 2004) and chemical nociception (Bandell *et al.* 2004; Jordt *et al.* 2004). Evaluation of transgenic animals lacking the protein dismissed an essential role of TRPA1 in hearing, but confirmed its major role in acute pain (Bautista *et al.* 2006; Kwan *et al.* 2006). Later studies also implicated TRPA1 in inflammatory and neuropathic pain (Dai *et al.* 2007; da Costa *et al.* 2010; Fernandes *et al.* 2011; Garrison & Stucky, 2014). Still, the specific role of TRPA1 in mammalian thermo- and mechanosensation has been the subject of some controversy (Chen *et al.* 2013; Moparthi *et al.* 2014), issues well covered in several reviews (Kwan & Corey, 2009; Zygmunt & Högestätt, 2014; Chen & Hackos, 2015). Although the canonical view describes mammalian TRPA1 channels as a noxious cold sensor (Story *et al.* 2003), a recent study reported a higher tolerance to noxious heat in TRPA1$^{-/-}$ mice (Hoffmann *et al.* 2013), suggesting that the thermosensory role of TRPA1 *in vivo* is far from settled. The characterization of TRPA1 orthologues in invertebrate and vertebrate species has contributed to a better understanding of the role of TRPA1 in thermosensation and chemosensation. These studies, combining analysis of chimeric and mutant channels, suggest a functional divergence of the protein and have been carefully reviewed recently (Laursen *et al.* 2014). Unlike the situation in mouse, in insects (e.g. *Drosophila melanogaster*, *Anopheles gambiae*) TRPA1 is activated by heat (Viswanath *et al.* 2003). Short isoforms lacking ankyrin repeats participate in mechanical nociception but are not involved in thermal nociception (Hwang *et al.* 2012; Zhong *et al.* 2012).

TRPA1 is found in a subset of somatic (dorsal root, trigeminal) and visceral (nodose) primary sensory neurons. Many TRPA1-expressing neurons are peptidergic nociceptors that co-express TRPV1 (Story *et al.* 2003). The local release of substance P or calcitonin gene-related peptide (CGRP) upon TRPA1 activation is an important part of its signalling mechanism, recruiting immune cells, producing vasodilatation and plasma extravasation and contributing to neurogenic inflammation (reviewed by Geppetti *et al.* 2008).

The structure of TRPA1 at near-atomic (~4 Å) resolution has been obtained by single-particle electron cryomicroscopy (cryo-EM) (Paulsen *et al.* 2015). This study, together with the cryo-EM structure of TRPV1 solved recently by the same group (Liao *et al.* 2013), represents an important milestone and will facilitate our understanding of TRPA1 function, including the characterization of molecular sites for drug interactions. A thorough discussion of structural features in TRPA1 has been published recently (Brewster & Gaudet, 2015). A graphical representation of TRPA1 structure and topology is shown in Fig. 1. The cryo-EM analysis confirmed that

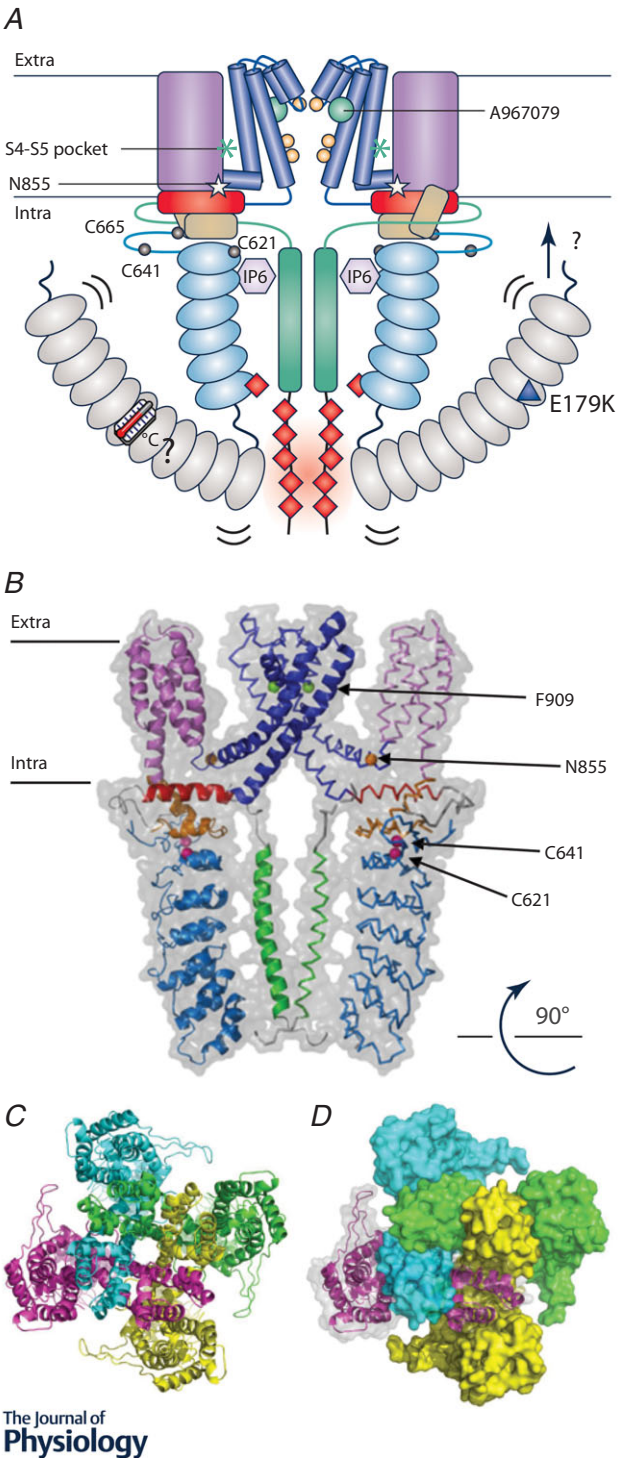



**Figure 1. Cryo-EM structure of human TRPA1**
*A*, schematic topology of two TRPA1 subunits based on the cryo-EM images with relevant functional regions shown in different colours. Ankyrin repeats (turquoise), pre-S1 linker (orange), S1–S4 (magenta), S5–S6 (blue), gate residues (orange), TRP-domain helix (red) and C-terminus coiled coil (green). The antagonist A-967079 is represented as a green circle and the S4–S5 pocket is marked with a green asterisk. Cysteines essential for response to reactive electrophiles (black circles); *myo*-inositol hexakisphosphate (IP6)

TRPA1 channels are tetrameric proteins formed by the assembly of four subunits (1119 amino acids in humans) with six transmembrane $\alpha$-helices (S1–S6). Notable elements in its structure include a series (14–18 depending on species) of ankyrin repeat domains (ARDs) within the long intracellular N-terminus. Despite suggestions for a role of these ARDs in thermosensation (see below), the evidence is still fragmentary. The cryo-EM images revealed an $\alpha$-helix directly after S6 with a structure analogous to the TRP domain in other TRP channels. The close proximity of the TRP-domain helix with non-contiguous structures such as the pre-S1 helix is consistent with a role in allosteric gating of the channel. Structural analysis revealed a binding pocket for A-967079, a selective TRPA1 antagonist, within S5–S6. It is notable that nearby residues are also involved in species-specific effects of menthol (activation/inhibition) (Xiao *et al.* 2008). Many structure and function studies helped identify key residues for agonist binding, ion permeation and subunit interactions. Some are highlighted in Fig. 2 with further details available in recently published reviews (Zygmunt & Högestätt, 2014; Brewster & Gaudet, 2015).

Several studies have characterized the permeation characteristics and ionic selectivity of TRPA1 (Karashima *et al.* 2010). At the structural level, the permeation pathway shows two major constriction sites (Paulsen *et al.* 2015). The channel is highly $Ca^{2+}$ permeable (Doerner *et al.* 2007; Zurborg *et al.* 2007) and permeating divalent cations contribute to channel regulation (Cordero-Morales *et al.* 2011; Sura *et al.* 2012), characterized by initial potentiation followed by desensitization (Wang *et al.* 2008*b*). This study identified an aspartate (D918 in mouse, D915 in human) within the pore loop critical for $Ca^{2+}$ permeability (Fig. 1). Divalent trace metals (i.e. $Zn^{2+}$, $Cu^{2+}$) also potentiate channel activity (Andersson *et al.* 2009; Hu *et al.* 2009) by interaction with residues in the C-terminus. TRPA1 channels have large pores that allow the permeation of large cationic molecules (Karashima *et al.* 2010). This feature has been exploited to incorporate large molecules such as lidocaine (lignocaine) into TRPA1 expressing axons to block their activity selectively (Roberson *et al.* 2013), a finding relevant for analgesia. Experiments reporting a dynamic alteration in ionic selectivity should be carefully scrutinized, in lieu of an alternative explanation of similar findings presented recently for P2X channels (Li *et al.* 2015). The biochemistry and pharmacology of the channel and its multifaceted behaviour and regulation have been the subject of comprehensive review articles in recent years (Viana & Ferrer-Montiel, 2009; Nilius *et al.* 2012; Bautista *et al.* 2013; Zygmunt & Högestätt, 2014; Chen & Hackos, 2015).

A key remaining question concerns the molecular mechanism of TRPA1 intrinsic thermosensitivity. Some studies are consistent with the idea of functional modules in the TRPA1 protein: replacement of these modules or mutations of specific residues should produce specific alterations in function. Chimeras between human and rattlesnake TRPA1 channels identified such a portable module for thermosensitivity within the ankyrin repeats of the N-terminus (Cordero-Morales *et al.* 2011). Similarly, a single mutation (S250N) within ankyrin 6 repeat in mouse TRPA1 was able to invert the sign of thermosensitivity, from cold to heat sensitive (Jabba *et al.* 2014). However, alterations in thermosensitivity have been identified in other locations, including the pore turret, S5 and S6 segments and the C terminus (Chen *et al.* 2013; Wang *et al.* 2013). An alternative proposal to portable functional modules to explain thermosensitivity is the hypothesis that it is a distributed function across different residues of the protein. Clapham and Miller presented a theoretical framework for this view based on the hypothesis that temperature-driven conformational rearrangements lead to changes in solvation (i.e. the hydration) of amino acid side chains (not necessarily clustered) which result in large changes in molar heat capacity ($\Delta C_P$) (Clapham & Miller, 2011). The solvation energy varies for polar and non-polar amino acids, affecting the sign of temperature sensitivity. In a remarkable empirical demonstration of this principle, Chowdhury and colleagues were able to design voltage-gated *Shaker* potassium channels with prominent temperature sensitivity (Chowdhury *et al.* 2014), a finding also reported on a separate study (Yang & Zheng, 2014). Considering that *Shaker* and TRPs belong to the same superfamily of ion channels, sharing many structural features, these observations should have a major impact on the analysis of thermosensitivity in TRP channels.

(grey hexagon). The position of the misense mutation N855S causing familial episodic pain syndrome is shown by the white star and the polymorphisms E179K causing reduced paradoxical heat sensation by the blue triangle. The N-terminus and ankyrin repeats 1–11, which are missing from the TRPA1 structure, are shown in grey. Curved black lines denote the suspected flexibility of the linkage between 1–11 and the remaining ankyrin repeats. Potential interactions between the N-terminus and the membrane or membrane-associated factors are indicated by a black arrow. Intracellular mutations reported to disturb calcium-dependent potentiation or inactivation of TRPA1 are marked with red diamonds. This figure, slightly modified from the original, is reprinted from Brewster & Gaudet (2015) with permission. *B*, ribbon and schematic diagram of human TRPA1 atomic model for residues Lys 446–Thr 1078 (Protein Data Bank entry 3J9P) viewed from the membrane plane. Only two subunits are presented, following the same colour scheme as in *A*. Residue F909 (green circle) interacts with the antagonist A-967079. Also shown are the locations of Cys 621 and 641 and Asn 855. *C*, schematic diagram of four TRPA1 subunits (each colour-coded) viewed from the extracellular face. *D*, same view of the surface with one the subunits partially visible to illustrate the domain-swapping of the S1–S4 bundle (lateral) and the pore-forming S5–S6 (medial) region.

## TRPA1 is a polymodal detector of danger signals

A unique aspect of TRPA1 function is its remarkable ligand promiscuity towards danger signals causing harm. Low and high temperature, osmotic challenges and a host of natural and industrial chemical irritants are known to activate TRPA1 channels (reviewed by Viana, 2011; Nilius *et al.* 2012; Zygmunt & Högestätt, 2014). Many TRPA1 activators are reactive electrophilic compounds. Electrophiles occur naturally in pungent or spicy plants such as allyl isothiocyanate (AITC) in wasabi, allicin in garlic or oleocanthal in extra virgin olive oil. TRPA1 activators include (*E*)-2-alkenals, aldehydes containing an unsaturated bond between the $\alpha$ and $\beta$ carbons, produced by many animals for chemical deterrence (Blair *et al.* 2016). The list of industrial electrophiles acting on TRPA1 is huge and includes aldehydes (e.g. formaldehyde, acetaldehyde), alkenals (e.g. acrolein, crotonaldehyde), hypochlorites, toluene diisocyanate and tear gases (reviewed by Bessac & Jordt, 2008). Channel gating of TRPA1 by electrophiles occurs by covalent modification of cysteine and lysine residues within the N-terminus (Hinman *et al.* 2006; Macpherson *et al.* 2007; Eberhardt *et al.* 2012) (Figs 1 and 2). There is no consensus about which specific cysteines, of a total of 28 in human TRPA1, are critical for gating by different electrophiles, although several are located near transmembrane segment S1 (Paulsen *et al.* 2015). The cryo-EM structure revealed the location of other cysteine and lysine residues in the transmembrane core and facing the lipid environment, which may be reactive with lipophilic electrophiles. Of note, activation of human TRPA1 by electrophilic compounds can occur in the absence of the first 688 amino acid residues (Moparthi *et al.* 2014), indicating the presence of additional sites important for activation.

TRPA1 can also be activated by non-reactive compounds that do not induce covalent modification, such as carvacrol, menthol, icilin, 2-aminoethoxydiphenyl borate (2-APB), thymol, nicotine, Δ-9-tetrahydrocannabinol and many others (reviewed by Viana & Ferrer-Montiel, 2009; Chen & Hackos, 2015). Additional activators of human TRPA1 include low pH (de la Roche *et al.* 2013)

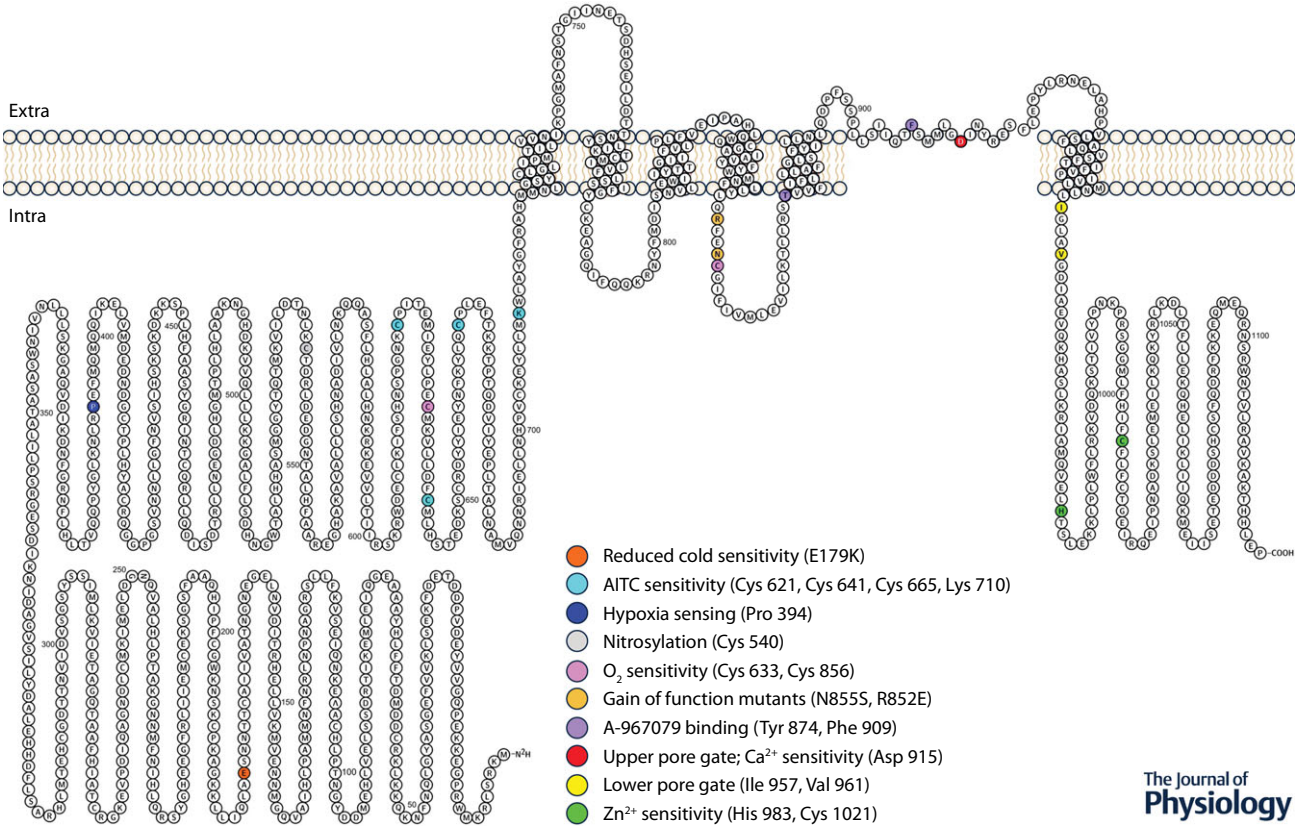



**Figure 2. Amino acid sequence of human TRPA1**
Each TRPA1 subunit (1119 amino acid residues) has a long intracellular $NH_2$ terminus (719 amino acids) characterized by multiple ankyrin domains, six transmembrane domains (S1–S6) and a shorter intracellular C-terminus. The figure highlights (colour coded) residues involved in TRPA1 function, including genetic variants linked to increased and decreased channel activity. Some residues important for AITC sensitivity are also involved in responses to other electrophilic agonists.

and intracellular alkalinization (Fujita *et al.* 2008), and polyunsaturated fatty acids (PUFAs) (Motter & Ahern, 2012). Moreover, a number of clinically relevant drugs, including general anaesthetics, aromatase inhibitors (used in the treatment of oestrogen receptor positive breast cancer), dihydropyridines and some non-steroidal anti-inflammatory drugs, are also activators of TRPA1, suggesting that the channel may play a role in their therapeutic actions and/or side effects (Fajardo *et al.* 2008; Andersson *et al.* 2011; Fusi *et al.* 2014; Kozai *et al.* 2015).

Activation of TRPA1 also occurs by signalling downstream of G protein-coupled receptors (GPCRs), a recognized mechanism for nociceptor sensitization (Bandell *et al.* 2004; Bautista *et al.* 2006; Wang *et al.* 2008*a*). GPCRs modulating TRPA1 include the bradykinin type 2 receptor, protease-activated receptor 2 and MAS-related GPCRs Mrgpr3 and MrgprC11, classically linked to phospholipase-C signalling (reviewed by Chen & Hackos, 2015). An additional role of protein kinase A in the sensitization mechanism of TRPA1 by bradykinin has been described (Wang *et al.* 2008*a*). Inorganic polyphosphates act as critical intracellular co-factors, sustaining TRPA1 activity (Kim & Cavanaugh, 2007). More specifically, *myo*-inositol hexakisphosphate (IP6) molecules have been identified in the cryo-EM structure of TRPA1, acting as a stabilizing bridge between the ARDs and the C-terminal coiled coil domain (Paulsen *et al.* 2015).

TRPA1 can form complexes with TRPV1 channels, altering their gating properties (reviewed by Akopian, 2011). Recently, this modulation was shown to be regulated by Tmen100, a membrane adaptor protein, resulting in major changes in TRPA1 activity (Weng *et al.* 2015). Plasma membrane translocation of channels in response to agonists is an additional modulatory mechanism of TRPA1 function (Schmidt *et al.* 2009).

## TRPA1 is a sensor of cellular stress, inflammation and tissue damage

Reactive oxygen species (ROS) are by-products of aerobic metabolism. They include superoxide anions ($\cdot O_2^-$), hydroxyl radical ($\cdot$OH) and hydrogen peroxide ($H_2O_2$). The main sources of $H_2O_2$ in cells are $\cdot O_2^-$ produced by mitochondria and cytoplasmic NADPH oxidases (NOXs). Cellular ROS levels depend on the fine balance between enzymatic cascades leading to ROS production or breakdown. Superoxide dismutases (SODs) convert $\cdot O_2^-$ enzymatically into $H_2O_2$. In contrast, the principal antioxidant redox enzymes belong to the glutathione-, catalase- and thioredoxin-dependent systems. In addition, antioxidant substances such as $\alpha$-tocopherol (vitamin E), $\beta$-carotene, ascorbate (vitamin C) and glutathione will affect redox homeostasis. ROS levels serve important signalling roles, including an adaptive response to stressful conditions (Schieber & Chandel, 2014). However, tissue damage can lead to an uncontrolled accumulation of ROS, a state known as oxidative stress, with major impact on cellular function (Droge, 2002).

TRPA1 acts as a sensor of toxic signals and molecular integrator of cellular stress, including ROS. Indeed, many known TRPA1 activators are algogenic substances released within the internal milieu in the course of inflammation or tissue injury. They include lipid peroxidation products such as 4-oxo-2-nonenal, 4-hydroxy-2-nonenal, 4-hydroxyhexenal (Trevisani *et al.* 2007) and oxidized lipids such as the cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$ (15-d-$PGJ_2$) (reviewed by Materazzi *et al.* 2008; Taylor-Clark *et al.* 2008; Chen & Hackos, 2015). Some activators of the inflammasome, such as monosodium urate (MSU) crystals, activate TRPA1 by an indirect mechanism involving production of $H_2O_2$ (Trevisan *et al.* 2014). Coupled to its expression pattern in barrier tissues, including the lung, the skin and the gut (see below), this broad activation profile makes TRPA1 an optimal cellular sensor for potential tissue damage. In the context of inflammation, ROS production can have beneficial, protective functions activating neutrophils and macrophages that serve an antimicrobial role (Droge, 2002; Schieber & Chandel, 2014). In this regard, TRPA1 appears to play a role in some of these protective functions, raising the important question of what level of TRPA1 activity is optimal for cell function.

Besides ROS, native and heterologously expressed TRPA1 channels are also activated by reactive nitrogen species (RNS), including nitric oxide (NO) and peroxynitrite ($ONOO^-$) (Miyamoto *et al.* 2009; Taylor-Clark *et al.* 2009*a*; Takahashi & Mori, 2011). These substances induce *S*-nitrosylation of TRPA1: the covalent attachment of NO to the sulphur atom of cysteine residues. Recently, streptozotocin, a toxin used to induce diabetes in animal models, was shown to activate TRPA1 by a mechanism dependent on oxidation by peroxynitrite (Andersson *et al.* 2015).

While many studies show a link between ROS or RNS elevation and TRPA1 activation, the mechanistic details are still faint. Furthermore, the relevance of this activation for cell signalling in specific tissues is unclear. In addition to direct channel gating, ROS and RNS products may affect signalling pathways or cellular redox state. Although a high concentration of $H_2O_2$ can activate TRPA1 (Andersson *et al.* 2008), other studies suggest an indirect activation mechanism by generation of lipid peroxidation products. Specifically, in cerebral endothelium, NOX2 is a major source of ROS. In this tissue, application of catalase, an enzyme that rapidly degrades $H_2O_2$, prevented NADPH-induced increases in TRPA1 activity (Sullivan *et al.* 2015). Using deferoxamine to chelate $Fe^{2+}$ and inhibit the Fenton reaction (i.e. the production of $\cdot$OH from $H_2O_2$ catalysed by $Fe^{2+}$),

the authors prevented NADPH-induced TRPA1 activity, suggesting that the effects of $H_2O_2$ are indirect and mediated by the formation of •OH and lipid peroxidation products (e.g. 4-hydroxy-2-nonenal).

High concentrations of hydrogen sulfide ($H_2S$) also activate TRPA1 and elicit pain (Andersson *et al.* 2012; Okubo *et al.* 2012). A recent study clarified this signalling pathway, linking the action of two endogenous gasotransmitters NO and $H_2S$ to the formation of nitroxyl anion (HNO), leading to TRPA1 activation, calcium influx and CGRP release, and the regulation of vascular tone (Eberhardt *et al.* 2014). Mass spectrometry analysis revealed disulphide formation between Cys 621 and the neighbouring Cys 633 as well as between Cys 651 and Cys 665 within the N-terminus of TRPA1. A follow-up study from the same group identified a similar mechanism in meningeal terminals of the trigeminal nerve, a finding relevant for migraine (Wild *et al.* 2015). Altogether, these studies questioned a direct activation of TRPA1 by NO or low (i.e. physiological) concentrations of $H_2S$.

In larval flies, TRPA1 activation by photochemical production of $H_2O_2$ participates in the detection of ultraviolet (UV) and blue light radiation by specific groups of neurons (Guntur *et al.* 2015). Interestingly, TRPA1 activation in human melanocytes is partially responsible for the increase in melanin production in response to UV light exposure (Bellono *et al.* 2013). In human melanocytes, the signalling pathway for UV activation of TRPA1 is specific for UV radiation, insensitive to the reducing agent dithiothreitol, insensitive to intracellular calcium levels and involves a retinal-dependent G protein and phospholipase C activation (Bellono *et al.* 2014).

TRPA1 has important functions in the lung and upper airways. Many vagal sensory endings respond to hypoxic conditions, resulting in cardiovascular and respiratory regulatory reflexes (Kubin *et al.* 2006). In vagal neurons of TRPA1$^{-/-}$ mice, responses to hyperoxia and mild hypoxia are abolished. TRPA1 is activated by hypoxia and hyperoxia by different molecular mechanisms (Takahashi *et al.* 2011). In normoxia, proline hydroxylation keeps the channel silent. Hypoxia reduces proline hydroxylase activity, leading to reduced hydroxylation of Pro 394 within the 10th ankyrin repeat domain, relieving inhibition. In contrast, hyperoxia (used clinically to treat hypoxaemia) leads to oxidation of Cys 633 and Cys 856 (Fig. 2). These results suggest that TRPA1 functions as a rapid alarm system during abnormal oxidative conditions. Acute mitochondrial dysfunction also leads to ROS generation and activation of TRPA1 in mouse bronchopulmonary C-fibres (Nesuashvili *et al.* 2013). This finding may be relevant to mechanisms of acute hypoxia sensing which also involve mitochondria-derived ROS (Fernandez-Aguera *et al.* 2015). $CO_2$ can also activate TRPA1 channels in trigeminal neurons, by a mechanism downstream of intracellular acidification (Wang *et al.* 2010).

Redox modulation may have some therapeutic potential in the context of TRPA1 dysfunction. For example, resveratrol, a natural stilbenoid with antioxidant and anti-inflammatory properties was shown to inhibit TRPA1 channels (Yu *et al.* 2013). Similarly, different antioxidants (*N*-acetyl-L-cysteine, lipoic acid and trolox) reduced TRPA1-dependent itch caused by oxidants (Liu & Ji, 2012) and ameliorated oxaliplatin-induced neuropathic pain (Ghirardi *et al.* 2005).

Abnormally low or high temperatures represent major cellular stress signals. Cold exposure triggers a vascular response in the skin, consisting of vasoconstriction followed by vasodilatation. This adjustment in blood supply is critical for protecting cutaneous tissues against cold injury. Recently, TRPA1 was shown to play a major role in this important vascular response (Aubdool *et al.* 2014).

Another example of the remarkable role of TRPA1 as a cellular integrator of harmful signals is exemplified by the effects of ciguatoxins. These polycyclic polyether toxins, produced by marine dinoflagellates, accumulate in large fish and are responsible for ciguatera poisoning. Symptoms in affected patients include peripheral sensory disturbances, often characterized by severe cold hypersensitivity. Pacific Ocean ciguatoxin 1 (P-CTX-1) does not activate TRPA1 channels directly but most TRPA1-expressing neurons are activated by P-CTX-1 (Vetter *et al.* 2012). When co-expressed with voltage-gated sodium channels, as occurs in sensory terminals, indirect activation of TRPA1 and additional effects of temperature (e.g. closure of leak $K^+$ channels) are sufficient to drive TRPA1-dependent calcium influx and nociceptor hyperexcitability that is responsible for the development of cold allodynia. In support of this interpretation, TRPA1$^{-/-}$ mice failed to develop ciguatoxin-induced cold allodynia and the excitatory effects of P-CTX-1 were strongly reduced in TRPA1-deficient nociceptive C-fibres. Another important finding in this study was the demonstration that TRPA1-dependent cold nociceptive pathways are activated at temperatures well above the noxious cold range, suggesting that these channels may play a broader role in protecting the body from damaging cold than previously recognized.

## TRPA1: a molecular sensor for harm beyond the boundaries of somatosensory neurons

TRPA1 was originally discovered in cultured human fibroblasts but its functional role in mesenchymal tissues was unknown at that time (Jaquemar *et al.* 1999). Early work on TRPA1 function focused on its sensory role within the peripheral nervous system (Story *et al.* 2003). However, the expression of TRPA1 in other tissues

(reviewed by Fernandes *et al.* 2012), including the vascular endothelium and all barrier tissues such as skin (e.g. keratinocytes, epithelial melanocytes), lung, gut, joint synoviocytes (Hatano *et al.* 2012) and cornea provides clues about a broader biological role of TRPA1 in tissue homeostasis.

Neurogenic vascular responses mediated by TRPA activation and neuropeptide release from sensory nerve endings are well established (Jordt *et al.* 2004; Geppetti *et al.* 2008; Eberhardt *et al.* 2014; Meseguer *et al.* 2014). However, accumulating evidence links non-neuronal TRPA1 activity to vascular control (reviewed by Earley, 2012). Endothelial TRPA1 mediates the vasodilatory response to TRPA1 agonists in cerebral arteries by a ROS-dependent mechanism (Earley *et al.* 2009; Sullivan *et al.* 2015). Pharmacological experiments ruled out the role of NO or prostacyclin in the response, but implicated activation of $Ca^{2+}$-activated $K^+$ channels, hyperpolarization and relaxation of smooth muscle cells (Sullivan *et al.* 2015). These studies suggest that endothelium TRPA1 plays a critical role in monitoring local oxidant and redox status in brain tissue, allowing precise regulation of vascular flow and nutrient availability (Sullivan *et al.* 2015). Interestingly, this functional arrangement was specific for brain cerebral arteries. However, in other tissues, TRPA1 expressed in peptidergic sensory terminals may play a similar functional role in vasomotor control (Jordt *et al.* 2004; Aubdool *et al.* 2014; Eberhardt *et al.* 2014; Meseguer *et al.* 2014).

TRPA1 agonists show functional activity in human skin keratinocytes and different types of fibroblasts (Atoyan *et al.* 2009; Tsutsumi *et al.* 2010), stimulating the release of inflammatory mediators. TRPA1 is also expressed in basal cells of the corneal epithelium. In chemically injured corneas, loss of TRPA1 expression, or the blockade of its activation with a selective antagonist, suppressed severe and persistent corneal inflammation, reducing fibrosis and scarring (Okada *et al.* 2014). The absence of TRPA1 was accompanied by reduced levels of transforming growth factor $\beta$1 (TGF-$\beta$1), interleukin 6 (IL-6), vascular endothelial growth factor and $\alpha$-1 type I collagen, markers of proinflammatory and profibrogenic activity. These studies show that, under certain conditions, TRPA1 activation can result in maladaptive responses such as tissue fibrosis.

Odontoblasts are tall columnar cells of neural crest origin that are part of the outermost layer of dental pulp and are responsible for dentin formation. Human odontoblasts express several TRP channels, including TRPA1 which may play a role in noxious cold responses in teeth (El Karim *et al.* 2011). Activation of TRPA1 channels in odontoblasts causes release of ATP to the extracellular medium, a possible excitatory mediator of dental pulp nociceptors (Egbuniwe *et al.* 2014).

Besides expression in C-fibre bladder afferents, TRPA1 is also found in urothelial cells, and its activation increases detrusor muscle contractions and micturition frequency (Streng *et al.* 2008; Gratzke *et al.* 2009). TRPA1 is expressed in rat and human colonic epithelia and is involved in electrogenic anion ($Cl^-$ and $HCO_3^-$) and fluid secretion, a mechanism important in host defence (Kaji *et al.* 2012). Human airway cells, including fibroblasts, epithelial and smooth muscle cells, express functional TRPA1 channels that can release interleukin 8 (IL-8) upon stimulation (Nassini *et al.* 2012). In human A549 lung carcinoma cells, activation of TRPA1 by low temperature or chemical agonists increased the production of NO.

The rather broad expression pattern of TRPA1, including pancreatic $\beta$ cells and brain astrocytes (Shigetomi *et al.* 2012), should be kept in mind when analysing the effects of pharmacological agents targeting TRPA1 and the potential adverse side effects of these drugs.

## TRPA1 and modulation of innate immunity

Pathogenic infection or tissue damage initiates a defence response that is intended to contain the spread of invading pathogens, neutralize their activity, clear damaged tissues and promote their repair. This immune response is triggered by the local release of viral products (e.g. RNA, envelope proteins) or pathogenic components of the bacterial wall, called pathogen-associated molecular patterns (PAMPs), which are recognized by pattern recognition receptors (PRRs) on leukocytes and macrophages and initiate an inflammatory response, including the release of cytokines and other mediators (e.g. NO) (Fig. 3). These PRRs include members of the Toll-like receptor (TLR) family (Janeway & Medzhitov, 2002), NOD-like receptors and RIG-I-like receptors. Similarly, tissue damage (e.g. mechanical wound, burn, freezing) also attracts and activates immune cells by certain intracellular products released from dying cells (Matzinger, 2002). These endogenous danger signals are known as alarmins or damage-associated molecular pattern (DAMP) molecules. DAMPs are structurally very diverse and include nuclear and cytosolic proteins, non-coding RNAs and small molecules (Bianchi, 2007). Examples of DAMPs are the high-mobility group box 1 protein, ATP, uric acid, hyaluronan fragments, heat shock proteins and let-7b miRNAs.

Pioneering work identified the role of vagal afferents in sensing proinflammatory cytokines (Hansen *et al.* 2001). Further studies over the past decade demonstrated an important bidirectional communication between the nervous system and the immune system, which has led to the suggestion that both are part of a unified host defence mechanism (Chiu *et al.* 2012) (Fig. 4). Infection or sterile tissue damage recruits immune and glial cells to

the site of injury. Activated immune cells release a variety of inflammatory mediators acting on multiple receptors on nociceptors, including TLRs and cytokine receptors, leading to their profound sensitization and exacerbated pain (Ren & Dubner, 2010) (Fig. 3). At the same time, the peripheral nervous system, including the autonomic nervous system, exerts a potent modulatory role of innate and adaptive immune responses (reviewed by Ordovas-Montanes *et al.* 2015). In part, the mechanism is dependent on antidromic axon reflexes in nociceptors that release neuropeptides, chemokines and other molecular mediators (e.g. NO).

Recent studies support a specific role for TRPA1 channels in the detection and response to harmful bacterial and viral products. The most compelling evidence comes from the discovery that TRPA1 is activated by lipopolysaccharide (LPS), or endotoxin, the main immunostimulant in Gram negative bacteria, causing the rapid activation of nociceptors (Meseguer *et al.* 2014). Moreover, activation of TRPA1 by LPS in vagal and somatic nociceptors led to the local release of neuropeptides (i.e. CGRP), causing pain, neurogenic inflammation and vasodilatation. Unlike traditional immune responses which are slow, these effects of toxic bacterial products are very fast, within seconds of their application, a rapid response arm in the host defence system. The molecular mechanisms leading to TRPA1 activation by LPS remain unclear. Activation did not involve TLR4, the canonical PRR for LPS (Poltorak *et al.* 1998). The authors found a correlation between structural features in lipid A, the biologically active lipid moiety in LPS, from different bacteria, TRPA1 activation *in vitro* and the capacity of

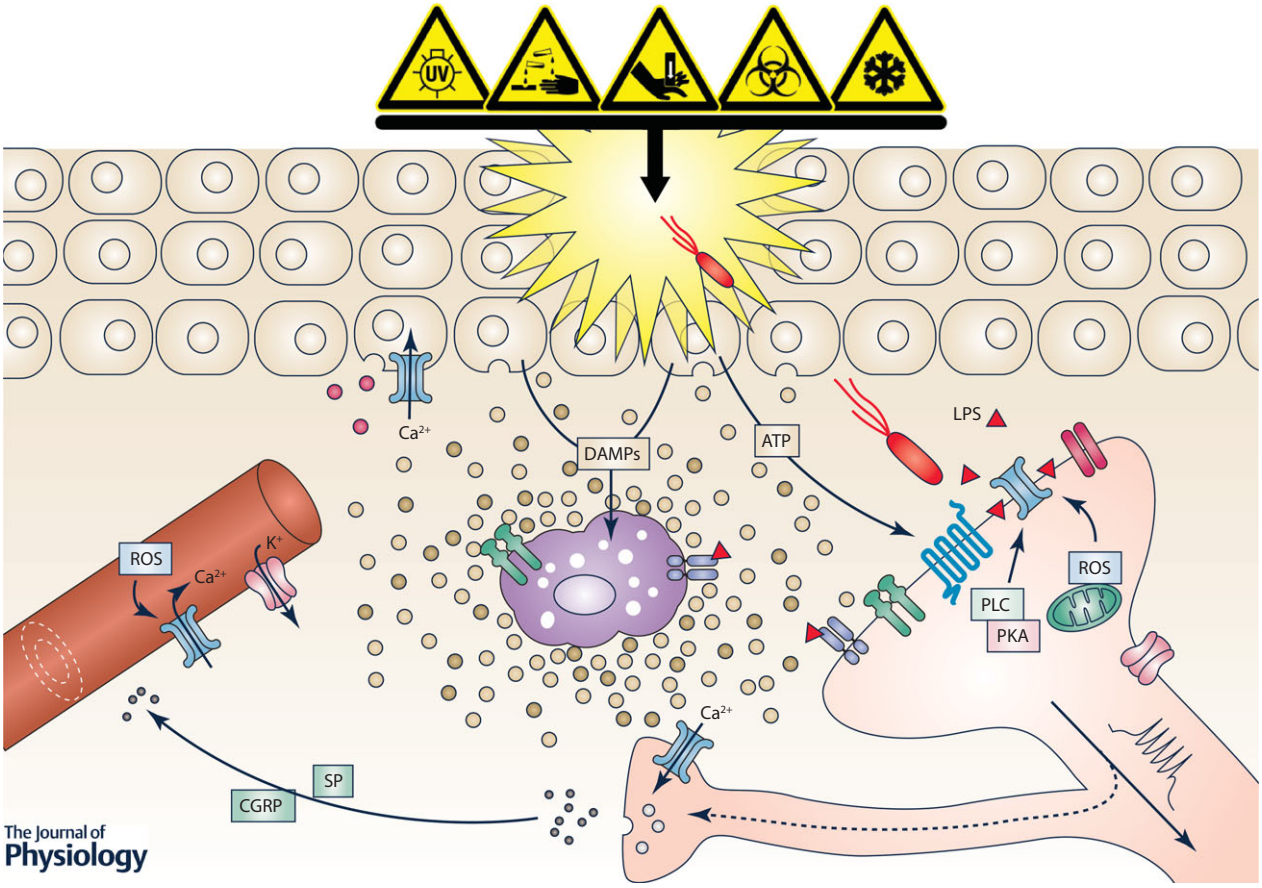



**Figure 3. TRPA1 is a sensor of danger signals in neuronal and non-neuronal tissues**
TRPA is expressed in peptidergic sensory nerve terminals and in different barrier tissues (e.g. skin keratinocytes, vascular endothelium). TRPA1 is activated by exogenous danger signals, including UV radiation, chemical irritants, bacterial products, mechanical forces and extreme temperatures. In addition, tissue damage releases intracellular alarmins such as ATP and uric acid that can signal to resident macrophages and extravasating monocytes. TRPA1 activation depolarizes nociceptor terminals sending pain signals to the CNS. The local calcium influx releases neuropeptides (e.g. substance P (SP) and CGRP) that act on the vasculature producing vasodilatation. In the brain, vasodilatation is linked to the opening of $Ca^{2+}$-activated potassium channels. Toxic bacterial products, including LPS, have rapid excitatory effects on TRPA1-expressing terminals. Inflammatory mediators act on membrane receptors (GPCRs, cytokines, TLRs) to sensitize nociceptive channels including TRPA1, resulting in further amplification of danger signals. TRPA1 is also activated by ROS and RNS.

different LPS classes to produce acute inflammation *in vivo*. In addition, trinitrophenol, an amphipatic molecule that causes membrane curvature, potentiated responses of TRPA1 channels to LPS (Meseguer *et al.* 2014). The authors speculated that LPS inserts in the bilayer, alters membrane tension and leads to TRPA1 opening. Other studies showed the direct activation of nociceptors by different bacterial products (e.g. *N*-formylated peptides) (Chiu *et al.* 2013). While a possible role of TRPA1 in these effects was not explored, it remains a distinct possibility. Of note, the activation of TRPA1 by PUFAs does not involve cysteines or known ligand binding domains of the channel (Motter & Ahern, 2012). I speculate that the mechanism involving activation of TRPA1 by PUFAs and lipid A may be similar. In another recent study, ablation of $Na_V1.8$-lineage neurons, which include the subpopulation of TRPA1(+) nociceptors, or pharmacological silencing of the same neurons, decreased eosinophilia and macrophage accumulation in broncheoalveolar lavage fluid following an allergen challenge (Talbot *et al.* 2015). This study demonstrates that nociceptors amplify pathological adaptive immune responses and that silencing these neurons with the anaesthetic QX-314 interrupts this neuroimmune interplay. Similarly, MSU crystals produce joint pain and inflammation by a TRPA1-dependent mechanism. Blockade of TRPA1 activity reduced neutrophil accumulation and proinflammatory cytokines (e.g. IL-1$\beta$) in the synovial fluid produced by MSU (Trevisan *et al.* 2014; see also Moilanen *et al.* 2015). Paradoxically, in human synoviocytes, TRPA1 activated by inflammatory mediators reduces cytokine (i.e. IL-6 and IL-8) release, acting as an anti-inflammatory

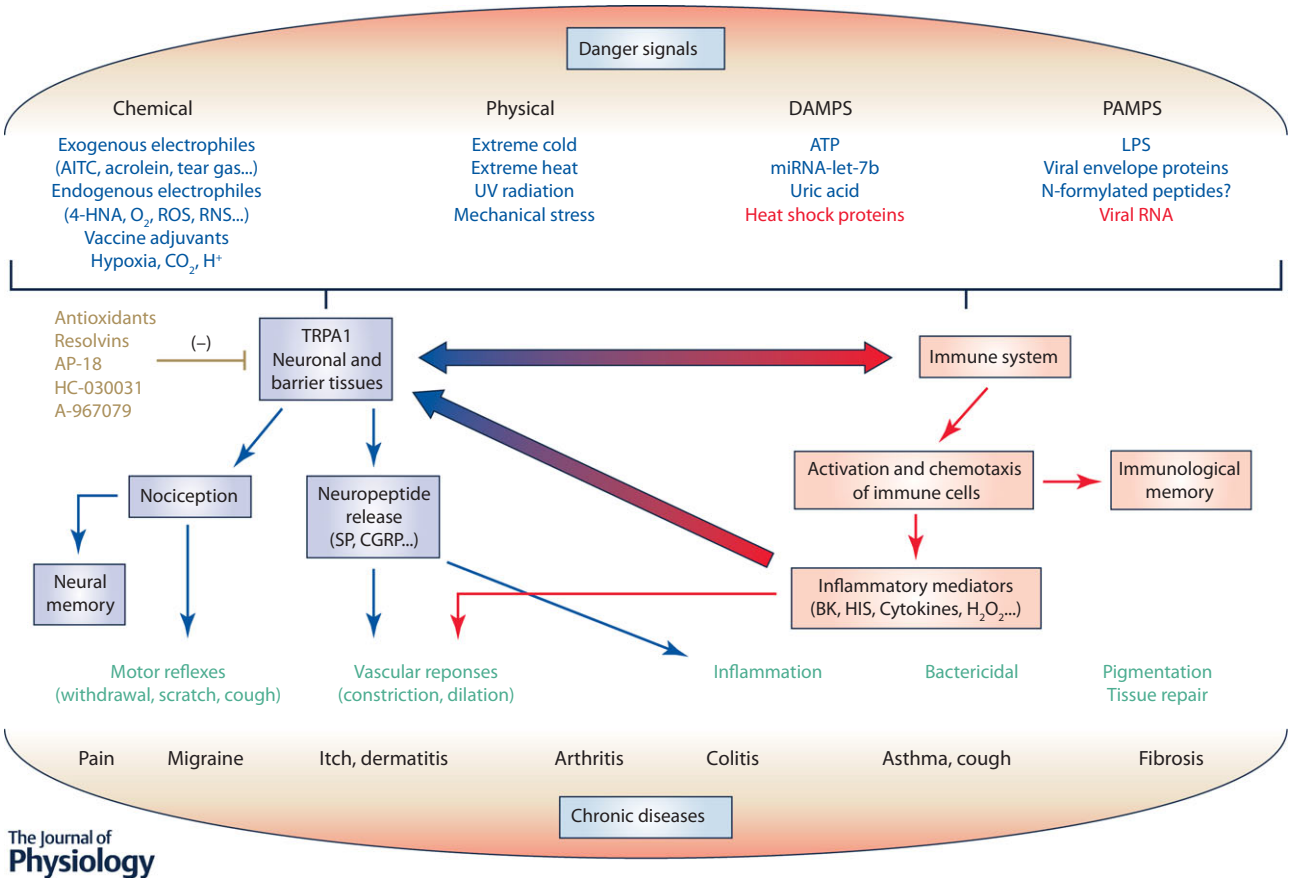



**Figure 4. Role of TRPA1 channels in neuroimmune interactions**
Non-neuronal and nociceptor TRPA1 channels respond to a very broad range of danger signals, including many molecules that activate host immunity. Listed in blue at the top of the figure are molecules for which a direct or indirect activation of TRPA1 has been demonstrated. Activation of immune cells releases inflammatory mediators that in many cases lead to sensitization of TRPA1-expressing nociceptors. This positive feedback loop can be further potentiated by the release of neuropeptides (SP, CGRP, etc.) from peptidergic nociceptors. The combined activation of the nociceptive and the immune system results in behavioural, vascular and tissue responses (listed in green) that minimize damage and promote repair. Alteration in the homeostatic balance between the nociceptive and the immune system can reinforce the response to danger signals, resulting in different chronic diseases. Inhibitors of TRPA1 activity (listed in orange) can minimize the response to danger signals exerting a negative bias against the exacerbation of inflammation and reducing pain.

feedback mechanism (Hatano *et al.* 2012). A recent article showed the rapid activation of TRPA1 channels by extracellular micro(mi)RNA let-7b in mouse dorsal root ganglion (DRG) neurons (Park *et al.* 2014). The effects required membrane-bound TLR7 but were independent of the canonical intracellular signalling mechanism for this receptor. Since miRNA let-7b can be released from DRG neurons in an activity-dependent manner, this may represent a novel mechanism for rapid DAMP-mediated nociceptor signalling. In another study, exposure of neuroblastoma cells to rhinovirus-derived soluble factors produced a rapid 50-fold increase in TRPA1 expression (Abdullah *et al.* 2014).

During the resolution phase of an acute inflammation, the immune system also releases soluble factors that promote tissue recovery, suppress inflammation and reduce pain. Among these endogenous factors there are several classes of lipid mediators including lipoxins, resolvins and neuroprotectins. Resolvins are derived from $\omega$-3 polyunsaturated fatty acids. Resolvins exert potent inhibition of TRPV1 and TRPA1 channels by G$\alpha$i-coupled GPCRs and reduce inflammatory pain (Bang *et al.* 2010; Park *et al.* 2011). These studies highlight the potential for pain inhibition by alternative signalling mechanisms targeting TRPA1.

Considering that the peripheral nervous system and the immune system represent the main sensory interfaces between the internal milieu and external environment, these findings underline TRPA1 as a critical player in danger detection and neuroimmune interactions (Fig. 4).

## Role of TRPA1 in the transition from acute to chronic pain

A fundamental and poorly understood aspect of pain pathophysiology is the transition from acute to chronic pain (Basbaum *et al.* 2009). It is a complex process that involves multiple mechanisms including recruitment of microglia, alterations in synaptic function and phenotypic alterations of nociceptors, including their peripheral sensitization (Reichling & Levine, 2009). TRPA1 channels play a major role in the mechanism of nociceptor sensitization. In this context, TRPA1 has been aptly labelled as a gatekeeper of inflammation (Bautista *et al.* 2013). Perhaps, a more accurate description is that of a dormant sentinel. Neuronal sensory circuits containing TRPA1-expressing endings appear to gate afferent input as a function of peripheral tissue status. This view may help explain some conflicting reports regarding mammalian TRPA1 function *in vivo*, for example in regard to its role in cold and mechanosensation. Under physiological conditions, inhibition or even ablation of 'dormant' TRPA1 neurons has a modest, or no influence, on cold sensitivity (Bautista *et al.* 2006; del Camino *et al.* 2010; Chen *et al.* 2011). Similarly, a deficit in acute mechanical nociception is not apparent in some studies of TRPA1$^{-/-}$ mice (Bautista *et al.* 2006) or after pharmacological blockade of the channel (Petrus *et al.* 2007; Trevisan *et al.* 2014). In contrast, in the context of inflammation (e.g. complete Freund's adjuvant (CFA) or MSU injection) or nerve injury, including oxaliplatin and paclitaxel-induced neuropathy, the contribution of TRPA1 to cold and mechanical allodynia is very prominent (Obata *et al.* 2005; Petrus *et al.* 2007; da Costa *et al.* 2010; del Camino *et al.* 2010; McGaraughty *et al.* 2010; Brierley *et al.* 2011; Chen *et al.* 2011; Lennertz *et al.* 2012; Materazzi *et al.* 2012; Trevisan *et al.* 2013, 2014).

Pharmacological blockade of TRPA1 reverses mechanical hypersensitivity in different models of inflammatory and neuropathic pain (Petrus *et al.* 2007; Eid *et al.* 2008; Lennertz *et al.* 2012). Moreover, TRPA1 antagonists can block the induction of inflammation and pain produced by algesic substances (Asgar *et al.* 2015), including chemotherapeutic agents (Nativi *et al.* 2013; Trevisan *et al.* 2013), and inflammasome activators (Trevisan *et al.* 2014). Similarly, A-967079, a selective TRPA1 antagonist, affected spontaneous activity or responses to low-intensity mechanical stimulation in spinal cord wide dynamic range neurons only under conditions of inflammation (McGaraughty *et al.* 2010). In mice, a single injection of CFA in the paw can lead to inflammatory arthritis and mechanical pain lasting for weeks. In young TRPA1$^{-/-}$ mice, development of mechanical hypersensitivity was delayed for at least 2 weeks, and in aged (2-year-old) mice it never took place (Garrison & Stucky, 2014), suggesting a major role of TRPA1 in this process. Similar results were observed in a different model of arthritis produced by MSU crystals (Trevisan *et al.* 2014).

Collectively, these results suggest that neural circuits with TRPA1-sensitized neurons modify their response to peripheral sensory input. The selective inhibition of sensitized responses with TRPA1 antagonists without altering normal sensitivity of mechanical and thermal afferents is of great therapeutic relevance. At which level the sensitization of TRPA1 is taking place is still unclear but an allosteric model of TRPA1 gating by different agonists predicts that a fraction of this potentiation is explained by the coupling of structurally independent sensors to the gating machinery of the channel (Salazar *et al.* 2011).

The role of TRPA1 in chronic pain may also depend on transcriptional regulatory mechanisms. Growth factors, including nerve growth factor, and muscle inflammation upregulate TRPA1 expression in trigeminal and DRG neurons (Diogenes *et al.* 2007; Asgar *et al.* 2015). Proinflammatory cytokines stimulate TRPA1 expression in synoviocytes by an NF-$\kappa$B signalling mechanism and downstream activation of HIF1$\alpha$ (Hatano *et al.* 2012). A similar induction of TRPA1 is produced by TGF-$\beta$1 in wounded corneal fibroblasts (Okada *et al.* 2014). In

rats, administration of the anti-tumour agent paclitaxel activated TLR4 receptors on satellite glial cells to cause release of TNF-$\alpha$, leading to increased expression of TRPA1 and TRPV4 in DRG neurons and neuropathic pain (Wu *et al.* 2015).

## TRPA1 and disease

The role of TRPA1 in disease states is expanding rapidly (Nilius *et al.* 2012). In some cases the link between a specific disease and TRPA1 function stems from animals models, while the evidence in human patients is still lacking. Early studies focused on its relation to cold and inflammatory pain, demonstrating a clear link between activation and/or upregulation of TRPA1 and inflammatory hypersensitivity and pain in different animal models. Later studies extended the findings to dental, postsurgical (Wei *et al.* 2012) and muscle pain (Asgar *et al.* 2015), and different models of arthritis (Trevisan *et al.* 2014) among others. In agreement with these findings, intracutaneous injection of the TRPA1 agonist cinnamaldehyde causes heat hyperalgesia in human volunteers (Namer *et al.* 2005). Similarly, intracutaneous injection of Angeli's salt, a precursor of HNO, a TRPA1 activator, evokes burning pain in humans (Eberhardt *et al.* 2014).

A gain of function mutation in the human *Trpa1* gene, located on chromosome 8q13, was identified in a Colombian family (Kremeyer *et al.* 2010). The disease, known as familial episodic pain syndrome-1, is an autosomal dominant disease characterized by the onset during infancy of episodic debilitating upper body pain episodes triggered by fasting, cold and physical stress. The genetic defect is a single missense mutation (N855S) in the intracellular S4–S5 linker segment (Fig. 2). Analysis of the mutant channel revealed a strong potentiation of agonist-evoked inward currents at physiological membrane potentials. Further exploration of the S4–S5 linker showed that the nearby R852E mutation leads to a gain of function and also suggested that charged residues E854 and K868 may form intersubunit salt bridges (Zima *et al.* 2015). Clinical evaluation of human TRPA1 polymorphisms identified a variant (E179K) associated with reduced paradoxical heat sensation, defined as a burning sensation when a noxious cold stimulus is applied (Binder *et al.* 2011). Subsequent functional analysis of this mutant revealed a reduced calcium response to prolonged cold (4ºC) exposure (May *et al.* 2012).

TRPA1 activity has been linked to the pathophysiology of other neurological diseases including migraine (Nassini *et al.* 2014) and peripheral neuropathies associated with diabetes and chemotherapy (Wei *et al.* 2009; Eberhardt *et al.* 2012). TRPA1 is also fundamental for the manifestations of some forms of itch (Lieu *et al.* 2014; Wilson & Bautista, 2014), a sensation that elicits stereotypical scratching, a protective response against cutaneous irritants and parasites. TRPA1 participates in other skin conditions, including allergic contact dermatitis (Oh *et al.* 2013).

Activation of nasal and lung nociceptors by inhaled environmental irritants provokes cough, bronchoconstriction and respiratory depression, defensive reflex reactions against harmful agents (e.g. dust, mites, chemical irritants, cold temperature). Numerous *in vivo* and *in vitro* studies have implicated TRPA1 in these protective reflexes (reviewed by Bessac & Jordt, 2008; Taylor-Clark *et al.* 2009*b*; Belvisi *et al.* 2011). Ovoalbumin-sensitized TRPA1$^{-/-}$ mice showed a diminished inflammatory infiltrate after the immunogen challenge as well as decreased airway hyper-reactivity (Caceres *et al.* 2009).

TRPA1 is involved in visceromotor responses to colorectal distension (Mueller-Tribbensee *et al.* 2015). It is strongly upregulated in experimental models of colitis in mice and rats and this upregulation is necessary for the development of visceral hyperalgesia (Yang *et al.* 2008). A similar upregulation was reported in patients with inflammatory bowel disease (IBD) (Kun *et al.* 2014). However, the role of TRPA1 in the pathogenesis of IBD is unclear. In some studies, activation of TRPA1(+) vagal sensory neurons has a proinflammatory effect in the gut by releasing substance P and the blockade of TRPA1 prevents colitis induction (Engel *et al.* 2011). In contrast, other studies reported a protective role of TRPA1 activation on the colonic inflammatory response (Kun *et al.* 2014).

Application of HC030031, a specific TRPA1 antagonist, led to a rise of blood pressure in rats, suggesting that TRPA1 activation plays a constitutive role in the regulation of blood pressure (Eberhardt *et al.* 2014). The interaction between $H_2S$ and endogenous NO produced HNO, activating TRPA1, $Ca^{2+}$ influx and triggering CGRP release. Of note, mice that lack the receptor for CGRP are spontaneously hypertensive. This study does not exclude TRPA1 effects other than at sensory endings.

A genome-wide DNA methylation analysis in pairs of identical twins with discordant heat pain sensitivity revealed differential methylation status within the TRPA1 promoter region. TRPA1 was hypermethylated (possibly implying reduced expression) in individuals with lower pain thresholds (Bell *et al.* 2014).

One of the most intriguing roles of TRPA1 channels has been unwrapped during the characterization of *Caenorhabditis elegans* mutants null for the *Trpa1* gene. These tiny worms, like many other animals, including mice (Conti *et al.* 2006), live longer in cold environments. Two recent studies suggest that lifespan in *C. elegans* is regulated by a signalling cascade involving cold-dependent activation of TRPA1, $Ca^{2+}$ influx and activation of

the transcription factor DAF-16/FOXO. Intriguingly, activation of this pathway extended the lifespan in adult worms (Xiao *et al.* 2013) but had the opposite outcome in larvae (Zhang *et al.* 2015).

### Therapeutic potential of TRPA1 modulators

The suggested role of TRPA1 in various diseases predicts an important therapeutic potential of its modulation and has triggered an intense search for specific blockers and their pre-clinical evaluation (Viana & Ferrer-Montiel, 2009; Jiang *et al.* 2011; Chen & Hackos, 2015). The selective antagonist CHEM-5861528, a derivative of HC-030031, alleviated mechanical hyperalgesia in a rat model of diabetic neuropathic pain (Wei *et al.* 2009). In osteoarthritic and inflamed rats, A-967079, a selective TRPA1 receptor antagonist, reduced activity to noxious mechanical stimulation in spinal cord wide dynamic range neurons (McGaraughty *et al.* 2010). The generation of monoclonal antibodies with antagonist activity towards human TRPA1 is another promising venue for the selective modulation of TRPA1 function, and a therapeutic alternative to small molecule screens already under way (Lee *et al.* 2014). Several compounds have species-specific modulatory effects on TRPA1. This should be kept in mind as a complicating factor when evaluating their therapeutic potential in humans.

Some surprises have emerged from the characterization of TRPA1 modulators. In a recent study, the anti-nociceptive action of acetaminophen, administered intrathecally, was attributed to TRPA1 activation in the superficial layers of the spinal dorsal horn (Andersson *et al.* 2011). The study showed that electrophilic metabolites of acetaminophen activated mouse and human TRPA1 and suggests the potential for spinal activation of TRPA1 in the treatment of pain. Consistent with this model, spinal application of AITC *in vivo* hyperpolarized substantia gelatinosa interneurons (Yamanaka *et al.* 2015).

### Summary, open questions and outlook

TRPA1 channels are ancient proteins, discovered only recently. TRPA1 is expressed in sensory and non-sensory cells in organisms spanning from worms to humans. Dissecting their various functional roles has followed a somewhat tortuous route and has led to truly exciting discoveries. The recent elucidation of TRPA1 structure will facilitate the dissection of its multiple functions and the identification of key residues for agonist and antagonist modulation.

TRPA1 acts as an integrator of endogenous and environmental harmful signals, including temperature, light (including UV radiation), bacterial toxins, mechanical forces and reactive chemicals. Activation of TRPA1 in sensory afferents can protect a host by promoting the avoidance of harmful environments (e.g. extreme temperatures, toxic agents, pathogenic microorganisms). Similarly, activation of TRPA1 in non-neuronal cells can also initiate protecting cascades, such as melanin production in the skin in response to solar UV radiation, fluid secretion in the gut and mucus production in the airways.

The peripheral nervous system and the immune system are functionally interconnected and there is an emerging view that TRP channels, including TRPA1, could play an important role in bridging both biological warning systems. How the input of TRPA1 afferents is gated at the level of the spinal cord and whether this gating is modified during chronic pain conditions is an important question that can be addressed with the combination of selective ablation, chemogenic silencing and optogenetic activation of specific afferents.

Defining the transcriptome of TRPA1 nociceptors under physiological and pathological conditions will provide a fingerprint for future investigations of possible functional partners. The epigenetic regulation of TRPA1 is another exciting aspect of nociceptor biology.

The link between TRPA1 and various diseases provides a rationale for considering its modulation a major druggable site for therapeutic intervention. Targeting molecular partners forming molecular complexes with TRPA1 represents a powerful alternative to direct modulation (Weng *et al.* 2015). However, the many protective functions of TRPA1 should also be kept in mind when designing clinical trials targeting its activity. For this reason, more basic research on TRPA1 functions is needed before TRPA1 modulators reach the clinic.

## References

Abdullah H, Heaney LG, Cosby SL & McGarvey LP (2014). Rhinovirus upregulates transient receptor potential channels in a human neuronal cell line: implications for respiratory virus-induced cough reflex sensitivity. *Thorax* **69**, 46–54.

Akopian AN (2011). Regulation of nociceptive transmission at the periphery via TRPA1-TRPV1 interactions. *Curr Pharm Biotechnol* **12**, 89–94.

Andersson DA, Filipovic MR, Gentry C, Eberhardt M, Vastani N, Leffler A, Reeh P & Bevan S (2015). Streptozotocin stimulates the ion channel TRPA1 directly: Involvement of peroxynitrite. *J Biol Chem* **290**, 15185–15196.

Andersson DA, Gentry C, Alenmyr L, Killander D, Lewis SE, Andersson A, Bucher B, Galzi JL, Sterner O, Bevan S, Högestätt ED & Zygmunt PM (2011). TRPA1 mediates spinal antinociception induced by acetaminophen and the cannabinoid $\Delta^9$-tetrahydrocannabiorcol. *Nat Commun* **2**, 551.

Andersson DA, Gentry C & Bevan S (2012). TRPA1 has a key role in the somatic pro-nociceptive actions of hydrogen sulfide. *PLoS One* **7**, e46917.

Andersson DA, Gentry C, Moss S & Bevan S (2008). Transient receptor potential A1 is a sensory receptor for multiple products of oxidative stress. *J Neurosci* **28**, 2485–2494.

Andersson DA, Gentry C, Moss S & Bevan S (2009). Clioquinol and pyrithione activate TRPA1 by increasing intracellular $Zn^{2+}$. *Proc Natl Acad Sci USA* **106**, 8374–8379.

Asgar J, Zhang Y, Saloman JL, Wang S, Chung MK & Ro JY (2015). The role of TRPA1 in muscle pain and mechanical hypersensitivity under inflammatory conditions in rats. *Neuroscience* **310**, 206–215.

Atoyan R, Shander D & Botchkareva NV (2009). Non-neuronal expression of transient receptor potential type A1 (TRPA1) in human skin. *J Invest Dermatol* **129**, 2312–2315.

Aubdool AA, Graepel R, Kodji X, Alawi KM, Bodkin JV, Srivastava S, Gentry C, Heads R, Grant AD, Fernandes ES, Bevan S & Brain SD (2014). TRPA1 is essential for the vascular response to environmental cold exposure. *Nat Commun* **5**, 5732.

Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, Earley TJ & Patapoutian A (2004). Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron* **41**, 849–857.

Bang S, Yoo S, Yang TJ, Cho H, Kim YG & Hwang SW (2010). Resolvin D1 attenuates activation of sensory transient receptor potential channels leading to multiple anti-nociception. *Br J Pharmacol* **161**, 707–720.

Basbaum AI, Bautista DM, Scherrer G & Julius D (2009). Cellular and molecular mechanisms of pain. *Cell* **139**, 267–284.

Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AI & Julius D (2006). TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. *Cell* **124**, 1269–1282.

Bautista DM, Pellegrino M & Tsunozaki M (2013). TRPA1: A gatekeeper for inflammation. *Annu Rev Physiol* **75**, 181–200.

Bell JT, Loomis AK, Butcher LM, Gao F, Zhang B, Hyde CL, Sun J, Wu H, Ward K, Harris J, Scollen S, Davies MN, Schalkwyk LC, Mill J, Mu TC, Williams FM, Li N, Deloukas P, Beck S, McMahon SB, Wang J, John SL & Spector TD (2014). Differential methylation of the TRPA1 promoter in pain sensitivity. *Nat Commun* **5**, 2978.

Bellono NW, Kammel LG, Zimmerman AL & Oancea E (2013). UV light phototransduction activates transient receptor potential A1 ion channels in human melanocytes. *Proc Natl Acad Sci USA* **110**, 2383–2388.

Bellono NW, Najera JA & Oancea E (2014). UV light activates a $G\alpha_{q/11}$-coupled phototransduction pathway in human melanocytes. *J Gen Physiol* **143**, 203–214.

Belvisi MG, Dubuis E & Birrell MA (2011). Transient receptor potential A1 channels: insights into cough and airway inflammatory disease. *Chest* **140**, 1040–1047.

Bessac BF & Jordt SE (2008). Breathtaking TRP channels: TRPA1 and TRPV1 in airway chemosensation and reflex control. *Physiology (Bethesda)* **23**, 360–370.

Bianchi ME (2007). DAMPs, PAMPs and alarmins: all we need to know about danger. *J Leukoc Biol* **81**, 1–5.

Binder A, May D, Baron R, Maier C, Tolle TR, Treede RD, Berthele A, Faltraco F, Flor H, Gierthmuhlen J, Haenisch S, Huge V, Magerl W, Maihofner C, Richter H, Rolke R, Scherens A, Uceyler N, Ufer M, Wasner G, Zhu J & Cascorbi I (2011). Transient receptor potential channel polymorphisms are associated with the somatosensory function in neuropathic pain patients. *PLoS One* **6**, e17387.

Blair NT, Philipson BI, Richards PM, Doerner JF, Segura A, Silver WL & Clapham DE (2016). Naturally produced defensive alkenal compounds activate TRPA1. *Chem Senses* **41**, 281–292.

Brewster MS & Gaudet R (2015). How the TRPA1 receptor transmits painful stimuli: Inner workings revealed by electron cryomicroscopy. *Bioessays* **37**, 1184–1192.

Brierley SM, Castro J, Harrington AM, Hughes PA, Page AJ, Rychkov GY & Blackshaw LA (2011). TRPA1 contributes to specific mechanically activated currents and sensory neuron mechanical hypersensitivity. *J Physiol* **589**, 3575–3593.

Caceres AI, Brackmann M, Elia MD, Bessac BF, del Camino D, D'Amours M, Witek JS, Fanger CM, Chong JA, Hayward NJ, Homer RJ, Cohn L, Huang X, Moran MM & Jordt SE (2009). A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma. *Proc Natl Acad Sci USA* **106**, 9099–9104.

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD & Julius D (1997). The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* **389**, 816–824.

Chen J & Hackos DH (2015). TRPA1 as a drug target – promise and challenges. *Naunyn Schmiedebergs Arch Pharmacol* **388**, 451–463.

Chen J, Joshi SK, DiDomenico S, Perner RJ, Mikusa JP, Gauvin DM, Segreti JA, Han P, Zhang XF, Niforatos W, Bianchi BR, Baker SJ, Zhong C, Simler GH, McDonald HA, Schmidt RG, McGaraughty SP, Chu KL, Faltynek CR, Kort ME, Reilly RM & Kym PR (2011). Selective blockade of TRPA1 channel attenuates pathological pain without altering noxious cold sensation or body temperature regulation. *Pain* **152**, 1165–1172.

Chen J, Kang D, Xu J, Lake M, Hogan JO, Sun C, Walter K, Yao B & Kim D (2013). Species differences and molecular determinant of TRPA1 cold sensitivity. *Nat Commun* **4**, 2501.

Chiu IM, Heesters BA, Ghasemlou N, Von Hehn CA, Zhao F, Tran J, Wainger B, Strominger A, Muralidharan S, Horswill AR, Bubeck Wardenburg J, Hwang SW, Carroll MC & Woolf CJ (2013). Bacteria activate sensory neurons that modulate pain and inflammation. *Nature* **501**, 52–57.

Chiu IM, von Hehn CA & Woolf CJ (2012). Neurogenic inflammation and the peripheral nervous system in host defense and immunopathology. *Nat Neurosci* **15**, 1063–1067.

Chowdhury S, Jarecki BW & Chanda B (2014). A molecular framework for temperature-dependent gating of ion channels. *Cell* **158**, 1148–1158.

Clapham DE & Miller C (2011). A thermodynamic framework for understanding temperature sensing by transient receptor potential (TRP) channels. *Proc Natl Acad Sci USA* **108**, 19492–19497.

Conti B, Sanchez-Alavez M, Winsky-Sommerer R, Morale MC, Lucero J, Brownell S, Fabre V, Huitron-Resendiz S, Henriksen S, Zorrilla EP, de Lecea L & Bartfai T (2006). Transgenic mice with a reduced core body temperature have an increased life span. *Science* **314**, 825–828.

Cordero-Morales JF, Gracheva EO & Julius D (2011). Cytoplasmic ankyrin repeats of transient receptor potential A1 (TRPA1) dictate sensitivity to thermal and chemical stimuli. *Proc Natl Acad Sci USA* **108**, E1184–1191.

Corey DP, Garcia-Anoveros J, Holt JR, Kwan KY, Lin SY, Vollrath MA, Amalfitano A, Cheung EL, Derfler BH, Duggan A, Geleoc GS, Grey PA, Hoffman MP, Rehm HL, Tamasauskas D & Zhang DS (2004). TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. *Nature* **432**, 723–730.

da Costa DS, Meotti FC, Andrade EL, Leal PC, Motta EM & Calixto JB (2010). The involvement of the transient receptor potential A1 (TRPA1) in the maintenance of mechanical and cold hyperalgesia in persistent inflammation. *Pain* **148**, 431–437.

Dai Y, Wang S, Tominaga M, Yamamoto S, Fukuoka T, Higashi T, Kobayashi K, Obata K, Yamanaka H & Noguchi K (2007). Sensitization of TRPA1 by PAR2 contributes to the sensation of inflammatory pain. *J Clin Invest* **117**, 1979–1987.

de la Roche J, Eberhardt MJ, Klinger AB, Stanslowsky N, Wegner F, Koppert W, Reeh PW, Lampert A, Fischer MJ & Leffler A (2013). The molecular basis for species-specific activation of human TRPA1 protein by protons involves poorly conserved residues within transmembrane domains 5 and 6. *J Biol Chem* **288**, 20280–20292.

del Camino D, Murphy S, Heiry M, Barrett LB, Earley TJ, Cook CA, Petrus MJ, Zhao M, D'Amours M, Deering N, Brenner GJ, Costigan M, Hayward NJ, Chong JA, Fanger CM, Woolf CJ, Patapoutian A & Moran MM (2010). TRPA1 contributes to cold hypersensitivity. *J Neurosci* **30**, 15165–15174.

Dhaka A, Viswanath V & Patapoutian A (2006). TRP ion channels and temperature sensation. *Annu Rev Neurosci* **29**, 135–161.

Diogenes A, Akopian AN & Hargreaves KM (2007). NGF up-regulates TRPA1: implications for orofacial pain. *J Dent Res* **86**, 550–555.

Doerner JF, Gisselmann G, Hatt H & Wetzel CH (2007). Transient receptor potential channel A1 is directly gated by calcium ions. *J Biol Chem* **282**, 13180–13189.

Droge W (2002). Free radicals in the physiological control of cell function. *Physiol Rev* **82**, 47–95.

Earley S (2012). TRPA1 channels in the vasculature. *Br J Pharmacol* **167**, 13–22.

Earley S, Gonzales AL & Crnich R (2009). Endothelium-dependent cerebral artery dilation mediated by TRPA1 and $Ca^{2+}$-activated $K^+$ channels. *Circ Res* **104**, 987–994.

Eberhardt M, Dux M, Namer B, Miljkovic J, Cordasic N, Will C, Kichko TI, de la Roche J, Fischer M, Suarez SA, Bikiel D, Dorsch K, Leffler A, Babes A, Lampert A, Lennerz JK, Jacobi J, Marti MA, Doctorovich F, Hogestatt ED, Zygmunt PM, Ivanovic-Burmazovic I, Messlinger K, Reeh P & Filipovic MR (2014). $H_2S$ and NO cooperatively regulate vascular tone by activating a neuroendocrine HNO-TRPA1-CGRP signalling pathway. *Nat Commun* **5**, 4381.

Eberhardt MJ, Filipovic MR, Leffler A, de la Roche J, Kistner K, Fischer MJ, Fleming T, Zimmermann K, Ivanovic-Burmazovic I, Nawroth PP, Bierhaus A, Reeh PW & Sauer SK (2012). Methylglyoxal activates nociceptors through transient receptor potential channel A1 (TRPA1): a possible mechanism of metabolic neuropathies. *J Biol Chem* **287**, 28291–28306.

Egbuniwe O, Grover S, Duggal AK, Mavroudis A, Yazdi M, Renton T, Di Silvio L & Grant AD (2014). TRPA1 and TRPV4 activation in human odontoblasts stimulates ATP release. *J Dent Res* **93**, 911–917.

Eid SR, Crown ED, Moore EL, Liang HA, Choong KC, Dima S, Henze DA, Kane SA & Urban MO (2008). HC-030031, a TRPA1 selective antagonist, attenuates inflammatory- and neuropathy-induced mechanical hypersensitivity. *Mol Pain* **4**, 48.

El Karim IA, Linden GJ, Curtis TM, About I, McGahon MK, Irwin CR, Killough SA & Lundy FT (2011). Human dental pulp fibroblasts express the 'cold-sensing' transient receptor potential channels TRPA1 and TRPM8. *J Endod* **37**, 473–478.

Engel MA, Leffler A, Niedermirtl F, Babes A, Zimmermann K, Filipovic MR, Izydorczyk I, Eberhardt M, Kichko TI, Mueller-Tribbensee SM, Khalil M, Siklosi N, Nau C, Ivanovic-Burmazovic I, Neuhuber WL, Becker C, Neurath MF & Reeh PW (2011). TRPA1 and substance P mediate colitis in mice. *Gastroenterology* **141**, 1346–1358.

Fajardo O, Meseguer V, Belmonte C & Viana F (2008). TRPA1 channels: novel targets of 1,4-dihydropyridines. *Channels (Austin)* **2**, 429–438.

Fernandes ES, Fernandes MA & Keeble JE (2012). The functions of TRPA1 and TRPV1: moving away from sensory nerves. *Br J Pharmacol* **166**, 510–521.

Fernandes ES, Russell FA, Spina D, McDougall JJ, Graepel R, Gentry C, Staniland AA, Mountford DM, Keeble JE, Malcangio M, Bevan S & Brain SD (2011). A distinct role for transient receptor potential ankyrin 1, in addition to transient receptor potential vanilloid 1, in tumor necrosis factor $\alpha$-induced inflammatory hyperalgesia and Freund's complete adjuvant-induced monarthritis. *Arthritis Rheum* **63**, 819–829.

Fernandez-Aguera MC, Gao L, Gonzalez-Rodriguez P, Pintado CO, Arias-Mayenco I, Garcia-Flores P, Garcia-Perganeda A, Pascual A, Ortega-Saenz P & Lopez-Barneo J (2015). Oxygen sensing by arterial chemoreceptors depends on mitochondrial complex I signaling. *Cell Metab* **22**, 825–837.

Fujita F, Uchida K, Moriyama T, Shima A, Shibasaki K, Inada H, Sokabe T & Tominaga M (2008). Intracellular alkalization causes pain sensation through activation of TRPA1 in mice. *J Clin Invest* **118**, 4049–4057.

Fusi C, Materazzi S, Benemei S, Coppi E, Trevisan G, Marone IM, Minocci D, De Logu F, Tuccinardi T, Di Tommaso MR, Susini T, Moneti G, Pieraccini G, Geppetti P & Nassini R (2014). Steroidal and non-steroidal third-generation aromatase inhibitors induce pain-like symptoms via TRPA1. *Nat Commun* **5**, 5736.

Garrison SR & Stucky CL (2014). Contribution of transient receptor potential ankyrin 1 to chronic pain in aged mice with complete Freund's adjuvant-induced arthritis. *Arthritis Rheumatol* **66**, 2380–2390.

Geppetti P, Nassini R, Materazzi S & Benemei S (2008). The concept of neurogenic inflammation. *BJU Int* **101**, Suppl. 3, 2–6.

Ghirardi O, Lo Giudice P, Pisano C, Vertechy M, Bellucci A, Vesci L, Cundari S, Miloso M, Rigamonti LM, Nicolini G, Zanna C & Carminati P (2005). Acetyl-L-carnitine prevents and reverts experimental chronic neurotoxicity induced by oxaliplatin, without altering its antitumor properties. *Anticancer Res* **25**, 2681–2687.

Gratzke C, Streng T, Waldkirch E, Sigl K, Stief C, Andersson KE & Hedlund P (2009). Transient receptor potential A1 (TRPA1) activity in the human urethra – evidence for a functional role for TRPA1 in the outflow region. *Eur Urol* **55**, 696–704.

Guntur AR, Gu P, Takle K, Chen J, Xiang Y & Yang CH (2015). *Drosophila* TRPA1 isoforms detect UV light via photochemical production of $H_2O_2$. *Proc Natl Acad Sci USA* **112**, E5753–E5761.

Hansen MK, O'Connor KA, Goehler LE, Watkins LR & Maier SF (2001). The contribution of the vagus nerve in interleukin-1$\beta$-induced fever is dependent on dose. *Am J Physiol Regul Integr Comp Physiol* **280**, R929–R934.

Hatano N, Itoh Y, Suzuki H, Muraki Y, Hayashi H, Onozaki K, Wood IC, Beech DJ & Muraki K (2012). Hypoxia-inducible factor-1$\alpha$ (HIF1$\alpha$) switches on transient receptor potential ankyrin repeat 1 (TRPA1) gene expression via a hypoxia response element-like motif to modulate cytokine release. *J Biol Chem* **287**, 31962–31972.

Hinman A, Chuang HH, Bautista DM & Julius D (2006). TRP channel activation by reversible covalent modification. *Proc Natl Acad Sci USA* **103**, 19564–19568.

Hoffmann T, Kistner K, Miermeister F, Winkelmann R, Wittmann J, Fischer MJ, Weidner C & Reeh PW (2013). TRPA1 and TRPV1 are differentially involved in heat nociception of mice. *Eur J Pain* **17**, 1472–1482.

Hu H, Bandell M, Petrus MJ, Zhu MX & Patapoutian A (2009). Zinc activates damage-sensing TRPA1 ion channels. *Nat Chem Biol* **5**, 183–190.

Hwang RY, Stearns NA & Tracey WD (2012). The ankyrin repeat domain of the TRPA protein painless is important for thermal nociception but not mechanical nociception. *PLoS One* **7**, e30090.

Jabba S, Goyal R, Sosa-Pagan JO, Moldenhauer H, Wu J, Kalmeta B, Bandell M, Latorre R, Patapoutian A & Grandl J (2014). Directionality of temperature activation in mouse TRPA1 ion channel can be inverted by single-point mutations in ankyrin repeat six. *Neuron* **82**, 1017–1031.

Janeway CA Jr & Medzhitov R (2002). Innate immune recognition. *Annu Rev Immunol* **20**, 197–216.

Jaquemar D, Schenker T & Trueb B (1999). An ankyrin-like protein with transmembrane domains is specifically lost after oncogenic transformation of human fibroblasts. *J Biol Chem* **274**, 7325–7333.

Jiang LH, Gamper N & Beech DJ (2011). Properties and therapeutic potential of transient receptor potential channels with putative roles in adversity: focus on TRPC5, TRPM2 and TRPA1. *Curr Drug Targets* **12**, 724–736.

Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Högestätt ED, Meng ID & Julius D (2004). Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature* **427**, 260–265.

Julius D (2013). TRP channels and pain. *Annu Rev Cell Dev Biol* **29**, 355–384.

Kaji I, Yasuoka Y, Karaki S & Kuwahara A (2012). Activation of TRPA1 by luminal stimuli induces EP4-mediated anion secretion in human and rat colon. *Am J Physiol Gastrointest Liver Physiol* **302**, G690–G701.

Karashima Y, Prenen J, Talavera K, Janssens A, Voets T & Nilius B (2010). Agonist-induced changes in $Ca^{2+}$ permeation through the nociceptor cation channel TRPA1. *Biophys J* **98**, 773–783.

Kim D & Cavanaugh EJ (2007). Requirement of a soluble intracellular factor for activation of transient receptor potential A1 by pungent chemicals: role of inorganic polyphosphates. *J Neurosci* **27**, 6500–6509.

Kozai D, Sakaguchi R, Ohwada T & Mori Y (2015). Deciphering subtype-selective modulations in TRPA1 biosensor channels. *Curr Neuropharmacol* **13**, 266–278.

Kremeyer B, Lopera F, Cox JJ, Momin A, Rugiero F, Marsh S, Woods CG, Jones NG, Paterson KJ, Fricker FR, Villegas A, Acosta N, Pineda-Trujillo NG, Ramirez JD, Zea J, Burley MW, Bedoya G, Bennett DL, Wood JN & Ruiz-Linares A (2010). A gain-of-function mutation in TRPA1 causes familial episodic pain syndrome. *Neuron* **66**, 671–680.

Kubin L, Alheid GF, Zuperku EJ & McCrimmon DR (2006). Central pathways of pulmonary and lower airway vagal afferents. *J Appl Physiol (1985)* **101**, 618–627.

Kun J, Szitter I, Kemeny A, Perkecz A, Kereskai L, Pohoczky K, Vincze A, Godi S, Szabo I, Szolcsanyi J, Pinter E & Helyes Z (2014). Upregulation of the transient receptor potential ankyrin 1 ion channel in the inflamed human and mouse colon and its protective roles. *PLoS One* **9**, e108164.

Kwan KY, Allchorne AJ, Vollrath MA, Christensen AP, Zhang DS, Woolf CJ & Corey DP (2006). TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. *Neuron* **50**, 277–289.

Kwan KY & Corey DP (2009). Burning cold: involvement of TRPA1 in noxious cold sensation. *J Gen Physiol* **133**, 251–256.

Laursen WJ, Bagriantsev SN & Gracheva EO (2014). TRPA1 channels: chemical and temperature sensitivity. *Curr Top Membr* **74**, 89–112.

Lee KJ, Wang W, Padaki R, Bi V, Plewa CA & Gavva NR (2014). Mouse monoclonal antibodies to transient receptor potential ankyrin 1 act as antagonists of multiple modes of channel activation. *J Pharmacol Exp Ther* **350**, 223–231.

Lennertz RC, Kossyreva EA, Smith AK & Stucky CL (2012). TRPA1 mediates mechanical sensitization in nociceptors during inflammation. *PLoS One* **7**, e43597.

Li M, Toombes GE, Silberberg SD & Swartz KJ (2015). Physical basis of apparent pore dilation of ATP-activated P2X receptor channels. *Nat Neurosci* **18**, 1577–1583.

Liao M, Cao E, Julius D & Cheng Y (2013). Structure of the TRPV1 ion channel determined by electron cryo-microscopy. *Nature* **504**, 107–112.

Lieu T, Jayaweera G, Zhao P, Poole DP, Jensen D, Grace M, McIntyre P, Bron R, Wilson YM, Krappitz M, Haerteis S, Korbmacher C, Steinhoff MS, Nassini R, Materazzi S, Geppetti P, Corvera CU & Bunnett NW (2014). The bile acid receptor TGR5 activates the TRPA1 channel to induce itch in mice. *Gastroenterology* **147**, 1417–1428.

Liu T & Ji RR (2012). Oxidative stress induces itch via activation of transient receptor potential subtype ankyrin 1 in mice. *Neurosci Bull* **28**, 145–154.

McGaraughty S, Chu KL, Perner RJ, Didomenico S, Kort ME & Kym PR (2010). TRPA1 modulation of spontaneous and mechanically evoked firing of spinal neurons in uninjured, osteoarthritic, and inflamed rats. *Mol Pain* **6**, 14.

Macpherson LJ, Dubin AE, Evans MJ, Marr F, Schultz PG, Cravatt BF & Patapoutian A (2007). Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. *Nature* **445**, 541–545.

Materazzi S, Fusi C, Benemei S, Pedretti P, Patacchini R, Nilius B, Prenen J, Creminon C, Geppetti P & Nassini R (2012). TRPA1 and TRPV4 mediate paclitaxel-induced peripheral neuropathy in mice via a glutathione-sensitive mechanism. *Pflugers Arch* **463**, 561–569.

Materazzi S, Nassini R, Andre E, Campi B, Amadesi S, Trevisani M, Bunnett NW, Patacchini R & Geppetti P (2008). Cox-dependent fatty acid metabolites cause pain through activation of the irritant receptor TRPA1. *Proc Natl Acad Sci USA* **105**, 12045–12050.

Matzinger P (2002). The danger model: a renewed sense of self. *Science* **296**, 301–305.

May D, Baastrup J, Nientit MR, Binder A, Schunke M, Baron R & Cascorbi I (2012). Differential expression and functionality of TRPA1 protein genetic variants in conditions of thermal stimulation. *J Biol Chem* **287**, 27087–27094.

Meseguer V, Alpizar YA, Luis E, Tajada S, Denlinger B, Fajardo O, Manenschijn JA, Fernandez-Pena C, Talavera A, Kichko T, Navia B, Sanchez A, Senaris R, Reeh P, Perez-Garcia MT, Lopez-Lopez JR, Voets T, Belmonte C, Talavera K & Viana F (2014). TRPA1 channels mediate acute neurogenic inflammation and pain produced by bacterial endotoxins. *Nat Commun* **5**, 3125.

Miyamoto T, Dubin AE, Petrus MJ & Patapoutian A (2009). TRPV1 and TRPA1 mediate peripheral nitric oxide-induced nociception in mice. *PLoS One* **4**, e7596.

Moilanen LJ, Hamalainen M, Lehtimaki L, Nieminen RM & Moilanen E (2015). Urate crystal induced inflammation and joint pain are reduced in transient receptor potential ankyrin 1 deficient mice – potential role for transient receptor potential ankyrin 1 in gout. *PLoS One* **10**, e0117770.

Moparthi L, Survery S, Kreir M, Simonsen C, Kjellbom P, Högestätt ED, Johanson U & Zygmunt PM (2014). Human TRPA1 is intrinsically cold- and chemosensitive with and without its N-terminal ankyrin repeat domain. *Proc Natl Acad Sci USA* **111**, 16901–16906.

Motter AL & Ahern GP (2012). TRPA1 is a polyunsaturated fatty acid sensor in mammals. *PLoS One* **7**, e38439.

Mueller-Tribbensee SM, Karna M, Khalil M, Neurath MF, Reeh PW & Engel MA (2015). Differential contribution of TRPA1, TRPV4 and TRPM8 to colonic nociception in mice. *PLoS One* **10**, e0128242.

Namer B, Seifert F, Handwerker HO & Maihofner C (2005). TRPA1 and TRPM8 activation in humans: effects of cinnamaldehyde and menthol. *Neuroreport* **16**, 955–959.

Nassini R, Materazzi S, Benemei S & Geppetti P (2014). The TRPA1 channel in inflammatory and neuropathic pain and migraine. *Rev Physiol Biochem Pharmacol* **167**, 1–43.

Nassini R, Pedretti P, Moretto N, Fusi C, Carnini C, Facchinetti F, Viscomi AR, Pisano AR, Stokesberry S, Brunmark C, Svitacheva N, McGarvey L, Patacchini R, Damholt AB, Geppetti P & Materazzi S (2012). Transient receptor potential ankyrin 1 channel localized to non-neuronal airway cells promotes non-neurogenic inflammation. *PLoS One* **7**, e42454.

Nativi C, Gualdani R, Dragoni E, Di Cesare Mannelli L, Sostegni S, Norcini M, Gabrielli G, la Marca G, Richichi B, Francesconi O, Moncelli MR, Ghelardini C & Roelens S (2013). A TRPA1 antagonist reverts oxaliplatin-induced neuropathic pain. *Sci Rep* **3**, 2005.

Nesuashvili L, Hadley SH, Bahia PK & Taylor-Clark TE (2013). Sensory nerve terminal mitochondrial dysfunction activates airway sensory nerves via transient receptor potential (TRP) channels. *Mol Pharmacol* **83**, 1007–1019.

Nilius B, Appendino G & Owsianik G (2012). The transient receptor potential channel TRPA1: from gene to pathophysiology. *Pflugers Arch* **464**, 425–458.

Obata K, Katsura H, Mizushima T, Yamanaka H, Kobayashi K, Dai Y, Fukuoka T, Tokunaga A, Tominaga M & Noguchi K (2005). TRPA1 induced in sensory neurons contributes to cold hyperalgesia after inflammation and nerve injury. *J Clin Invest* **115**, 2393–2401.

Oh MH, Oh SY, Lu J, Lou H, Myers AC, Zhu Z & Zheng T (2013). TRPA1-dependent pruritus in IL-13-induced chronic atopic dermatitis. *J Immunol* **191**, 5371–5382.

Okada Y, Shirai K, Reinach PS, Kitano-Izutani A, Miyajima M, Flanders KC, Jester JV, Tominaga M & Saika S (2014). TRPA1 is required for TGF-$\beta$ signaling and its loss blocks inflammatory fibrosis in mouse corneal stroma. *Lab Invest* **94**, 1030–1041.

Okubo K, Matsumura M, Kawaishi Y, Aoki Y, Matsunami M, Okawa Y, Sekiguchi F & Kawabata A (2012). Hydrogen sulfide-induced mechanical hyperalgesia and allodynia require activation of both $Ca_v3.2$ and TRPA1 channels in mice. *Br J Pharmacol* **166**, 1738–1743.

Ordovas-Montanes J, Rakoff-Nahoum S, Huang S, Riol-Blanco L, Barreiro O & von Andrian UH (2015). The regulation of immunological processes by peripheral neurons in homeostasis and disease. *Trends Immunol* **36**, 578–604.

Park CK, Xu ZZ, Berta T, Han Q, Chen G, Liu XJ & Ji RR (2014). Extracellular microRNAs activate nociceptor neurons to elicit pain via TLR7 and TRPA1. *Neuron* **82**, 47–54.

Park CK, Xu ZZ, Liu T, Lu N, Serhan CN & Ji RR (2011). Resolvin D2 is a potent endogenous inhibitor for transient receptor potential subtype V1/A1, inflammatory pain, and spinal cord synaptic plasticity in mice: distinct roles of resolvin D1, D2, and E1. *J Neurosci* **31**, 18433–18438.

Paulsen CE, Armache JP, Gao Y, Cheng Y & Julius D (2015). Structure of the TRPA1 ion channel suggests regulatory mechanisms. *Nature* **525**, 552.

Petrus M, Peier AM, Bandell M, Hwang SW, Huynh T, Olney N, Jegla T & Patapoutian A (2007). A role of TRPA1 in mechanical hyperalgesia is revealed by pharmacological inhibition. *Mol Pain* **3**, 40.

Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B & Beutler B (1998). Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science* **282**, 2085–2088.

Reichling DB & Levine JD (2009). Critical role of nociceptor plasticity in chronic pain. *Trends Neurosci* **32**, 611–618.

Ren K & Dubner R (2010). Interactions between the immune and nervous systems in pain. *Nat Med* **16**, 1267–1276.

Roberson DP, Gudes S, Sprague JM, Patoski HA, Robson VK, Blasl F, Duan B, Oh SB, Bean BP, Ma Q, Binshtok AM & Woolf CJ (2013). Activity-dependent silencing reveals functionally distinct itch-generating sensory neurons. *Nat Neurosci* **16**, 910–918.

Salazar M, Moldenhauer H & Baez-Nieto D (2011). Could an allosteric gating model explain the role of TRPA1 in cold hypersensitivity? *J Neurosci* **31**, 5554–5556.

Schieber M & Chandel NS (2014). ROS function in redox signaling and oxidative stress. *Curr Biol* **24**, R453–R462.

Schmidt M, Dubin AE, Petrus MJ, Earley TJ & Patapoutian A (2009). Nociceptive signals induce trafficking of TRPA1 to the plasma membrane. *Neuron* **64**, 498–509.

Shigetomi E, Tong X, Kwan KY, Corey DP & Khakh BS (2012). TRPA1 channels regulate astrocyte resting calcium and inhibitory synapse efficacy through GAT-3. *Nat Neurosci* **15**, 70–80.

Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, Hricik TR, Earley TJ, Hergarden AC, Andersson DA, Hwang SW, McIntyre P, Jegla T, Bevan S & Patapoutian A (2003). ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell* **112**, 819–829.

Streng T, Axelsson HE, Hedlund P, Andersson DA, Jordt SE, Bevan S, Andersson KE, HögestĠtt ED & Zygmunt PM (2008). Distribution and function of the hydrogen sulfide-sensitive TRPA1 ion channel in rat urinary bladder. *Eur Urol* **53**, 391–399.

Sullivan MN, Gonzales AL, Pires PW, Bruhl A, Leo MD, Li W, Oulidi A, Boop FA, Feng Y, Jaggar JH, Welsh DG & Earley S (2015). Localized TRPA1 channel $Ca^{2+}$ signals stimulated by reactive oxygen species promote cerebral artery dilation. *Sci Signal* **8**, ra2.

Sura L, Zima V, Marsakova L, Hynkova A, Barvik I & Vlachova V (2012). C-terminal acidic cluster is involved in $Ca^{2+}$-induced regulation of human transient receptor potential ankyrin 1 channel. *J Biol Chem* **287**, 18067–18077.

Takahashi N, Kuwaki T, Kiyonaka S, Numata T, Kozai D, Mizuno Y, Yamamoto S, Naito S, Knevels E, Carmeliet P, Oga T, Kaneko S, Suga S, Nokami T, Yoshida J & Mori Y (2011). TRPA1 underlies a sensing mechanism for $O_2$. *Nat Chem Biol* **7**, 701–711.

Takahashi N & Mori Y (2011). TRP channels as sensors and signal integrators of redox status changes. *Front Pharmacol* **2**, 58.

Talbot S, Abdulnour RE, Burkett PR, Lee S, Cronin SJ, Pascal MA, Laedermann C, Foster SL, Tran JV, Lai N, Chiu IM, Ghasemlou N, DiBiase M, Roberson D, Von Hehn C, Agac B, Haworth O, Seki H, Penninger JM, Kuchroo VK, Bean BP, Levy BD & Woolf CJ (2015). Silencing nociceptor neurons reduces allergic airway inflammation. *Neuron* **87**, 341–354.

Taylor-Clark TE, Ghatta S, Bettner W & Undem BJ (2009*a*). Nitrooleic acid, an endogenous product of nitrative stress, activates nociceptive sensory nerves via the direct activation of TRPA1. *Mol Pharmacol* **75**, 820–829.

Taylor-Clark TE, Nassenstein C, McAlexander MA & Undem BJ (2009*b*). TRPA1: a potential target for anti-tussive therapy. *Pulm Pharmacol Ther* **22**, 71–74.

Taylor-Clark TE, Undem BJ, Macglashan DW Jr, Ghatta S, Carr MJ & McAlexander MA (2008). Prostaglandin-induced activation of nociceptive neurons via direct interaction with transient receptor potential A1 (TRPA1). *Mol Pharmacol* **73**, 274–281.

Thakur M, Crow M, Richards N, Davey GI, Levine E, Kelleher JH, Agley CC, Denk F, Harridge SD & McMahon SB (2014). Defining the nociceptor transcriptome. *Front Mol Neurosci* **7**, 87.

Trevisan G, Hoffmeister C, Rossato MF, Oliveira SM, Silva MA, Silva CR, Fusi C, Tonello R, Minocci D, Guerra GP, Materazzi S, Nassini R, Geppetti P & Ferreira J (2014). TRPA1 receptor stimulation by hydrogen peroxide is critical to trigger hyperalgesia and inflammation in a model of acute gout. *Free Radic Biol Med* **72**, 200–209.

Trevisan G, Materazzi S, Fusi C, Altomare A, Aldini G, Lodovici M, Patacchini R, Geppetti P & Nassini R (2013). Novel therapeutic strategy to prevent chemotherapy-induced persistent sensory neuropathy by TRPA1 blockade. *Cancer Res* **73**, 3120–3131.

Trevisani M, Siemens J, Materazzi S, Bautista DM, Nassini R, Campi B, Imamachi N, Andre E, Patacchini R, Cottrell GS, Gatti R, Basbaum AI, Bunnett NW, Julius D & Geppetti P (2007). 4-Hydroxynonenal, an endogenous aldehyde, causes pain and neurogenic inflammation through activation of the irritant receptor TRPA1. *Proc Natl Acad Sci USA* **104**, 13519–13524.

Tsutsumi M, Denda S, Ikeyama K, Goto M & Denda M (2010). Exposure to low temperature induces elevation of intracellular calcium in cultured human keratinocytes. *J Invest Dermatol* **130**, 1945–1948.

Vetter I, Touska F, Hess A, Hinsbey R, Sattler S, Lampert A, Sergejeva M, Sharov A, Collins LS, Eberhardt M, Engel M, Cabot PJ, Wood JN, Vlachova V, Reeh PW, Lewis RJ & Zimmermann K (2012). Ciguatoxins activate specific cold pain pathways to elicit burning pain from cooling. *EMBO J* **31**, 3795–3808.

Viana F (2011). Chemosensory properties of the trigeminal system. *ACS Chem Neurosci* **2**, 38–50.

Viana F & Ferrer-Montiel A (2009). TRPA1 modulators in pre-clinical development. *Expert Opin Ther Pat* **19**, 1787–1799.

Viswanath V, Story GM, Peier AM, Petrus MJ, Lee VM, Hwang SW, Patapoutian A & Jegla T (2003). Opposite thermosensor in fruitfly and mouse. *Nature* **423**, 822–823.

Wang H, Schupp M, Zurborg S & Heppenstall PA (2013). Residues in the pore region of *Drosophila* transient receptor potential A1 dictate sensitivity to thermal stimuli. *J Physiol* **591**, 185–201.

Wang S, Dai Y, Fukuoka T, Yamanaka H, Kobayashi K, Obata K, Cui X, Tominaga M & Noguchi K (2008*a*). Phospholipase C and protein kinase A mediate bradykinin sensitization of TRPA1: a molecular mechanism of inflammatory pain. *Brain* **131**, 1241–1251.

Wang YY, Chang RB & Liman ER (2010). TRPA1 is a component of the nociceptive response to $CO_2$. *J Neurosci* **30**, 12958–12963.

Wang YY, Chang RB, Waters HN, McKemy DD & Liman ER (2008*b*). The nociceptor ion channel TRPA1 is potentiated and inactivated by permeating calcium ions. *J Biol Chem* **283**, 32691–32703.

Wei H, Hamalainen MM, Saarnilehto M, Koivisto A & Pertovaara A (2009). Attenuation of mechanical hypersensitivity by an antagonist of the TRPA1 ion channel in diabetic animals. *Anesthesiology* **111**, 147–154.

Wei H, Karimaa M, Korjamo T, Koivisto A & Pertovaara A (2012). Transient receptor potential ankyrin 1 ion channel contributes to guarding pain and mechanical hypersensitivity in a rat model of postoperative pain. *Anesthesiology* **117**, 137–148.

Weng HJ, Patel KN, Jeske NA, Bierbower SM, Zou W, Tiwari V, Zheng Q, Tang Z, Mo GC, Wang Y, Geng Y, Zhang J, Guan Y, Akopian AN & Dong X (2015). Tmem100 is a regulator of TRPA1-TRPV1 complex and contributes to persistent pain. *Neuron* **85**, 833–846.

Wild V, Messlinger K & Fischer MJ (2015). Hydrogen sulfide determines HNO-induced stimulation of trigeminal afferents. *Neurosci Lett* **602**, 104–109.

Wilson SR & Bautista DM (2014). Role of transient receptor potential channels in acute and chronic itch. In *Itch: Mechanisms and Treatment*, ed. Carstens E & Akiyama T. CRC Press, Boca Raton, FL, USA.

Woolf CJ & Ma Q (2007). Nociceptors – noxious stimulus detectors. *Neuron* **55**, 353–364.

Wu Z, Wang S, Wu I, Mata M & Fink DJ (2015). Activation of TLR-4 to produce tumour necrosis factor-$\alpha$ in neuropathic pain caused by paclitaxel. *Eur J Pain* **19**, 889–898.

Xiao B, Dubin AE, Bursulaya B, Viswanath V, Jegla TJ & Patapoutian A (2008). Identification of transmembrane domain 5 as a critical molecular determinant of menthol sensitivity in mammalian TRPA1 channels. *J Neurosci* **28**, 9640–9651.

Xiao R, Zhang B, Dong Y, Gong J, Xu T, Liu J & Xu XZ (2013). A genetic program promotes *C. elegans* longevity at cold temperatures via a thermosensitive TRP channel. *Cell* **152**, 806–817.

Yamanaka M, Taniguchi W, Nishio N, Hashizume H, Yamada H, Yoshida M & Nakatsuka T (2015). In vivo patch-clamp analysis of the antinociceptive actions of TRPA1 activation in the spinal dorsal horn. *Mol Pain* **11**, 20.

Yang F & Zheng J (2014). High temperature sensitivity is intrinsic to voltage-gated potassium channels. *Elife* **3**, e03255.

Yang J, Li Y, Zuo X, Zhen Y, Yu Y & Gao L (2008). Transient receptor potential ankyrin-1 participates in visceral hyperalgesia following experimental colitis. *Neurosci Lett* **440**, 237–241.

Yu L, Wang S, Kogure Y, Yamamoto S, Noguchi K & Dai Y (2013). Modulation of TRP channels by resveratrol and other stilbenoids. *Mol Pain* **9**, 3.

Zhang B, Xiao R, Ronan EA, He Y, Hsu AL, Liu J & Xu XZ (2015). Environmental temperature differentially modulates *C. elegans* longevity through a thermosensitive TRP channel. *Cell Rep* **11**, 1414–1424.

Zhong L, Bellemer A, Yan H, Ken H, Jessica R, Hwang RY, Pitt GS & Tracey WD (2012). Thermosensory and nonthermosensory isoforms of *Drosophila melanogaster* TRPA1 reveal heat-sensor domains of a thermoTRP channel. *Cell Rep* **1**, 43–55.

Zima V, Witschas K, Hynkova A, Zimova L, Barvik I & Vlachova V (2015). Structural modeling and patch-clamp analysis of pain-related mutation TRPA1-N855S reveal inter-subunit salt bridges stabilizing the channel open state. *Neuropharmacology* **93**, 294–307.

Zurborg S, Yurgionas B, Jira JA, Caspani O & Heppenstall PA (2007). Direct activation of the ion channel TRPA1 by $Ca^{2+}$. *Nat Neurosci* **10**, 277–279.

Zygmunt PM & Högestätt ED (2014). TRPA1. *Handb Exp Pharmacol* **222**, 583–630.

## Additional information

### Competing interests

None declared.

### Funding

The authors' work is supported by funds from Spanish MINECO projects SAF2013-45608-R and CONSOLIDER-INGENIO 2010 CSD2007-0002 and Generalitat Valenciana Prometeo/2014/011. Instituto de Neurociencias de Alicante is a Severo Ochoa Excellence Centre.

### Acknowledgements

I am grateful to current and previous lab members for their constant effort and fruitful discussions. I thank Rosa Señarís for comments, Stuart Ingham for professional help with illustrations and Alfonso Martinez de la Cruz for advice with PyMol molecular visualization software.