Pharmacology & Therapeutics 200 (2019) 13–26

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Photopharmacology and opto-chemogenetics of TRPC channels-some therapeutic visions

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ARTICLE INFO

Keywords:
TRPC channels
Photopharmacology
Photochromic ligands
Chemogenetic manipulation
TRPC pathophysiology

ABSTRACT

Non-selective cation conductances formed by transient receptor potential canonical (TRPC) proteins govern the function and fate of a wide range of human cell types. In the past decade, evidence has accumulated for a pivotal role of these channels in human diseases, raising substantial interest in their therapeutic targeting. As yet, an appreciable number of small molecules for block and modulation of recombinant TRPC conductances have been identified. However, groundbreaking progress in TRPC pharmacology towards therapeutic applications is lagging behind due to incomplete understanding of their molecular pharmacology and their exact role in disease. A major breakthrough that is expected to overcome these hurdles is the recent success in obtaining high-resolution structure information on TRPC channel complexes and the advent of TRP photopharmacology and optogenetics. Here, we summarize current concepts of enhancing the precision of therapeutic interference with TRPC signaling and TRPC-mediated pathological processes.

Contents

1. Introduction - developments in TRPC pharmacology

Identification of mammalian homologues of the drosophila *trp* gene product (Nilius & Flockerzi, 2014; Zhu et al., 1996), which were originally recognized as key players in the insects' visual transduction cascade (Minke & Cook, 2002), initiated intensive research to uncover the role of these proteins in mammalian and human physiopathology. Members of the canonical subfamily (TRPC), which are closely related to the drosophila *trp* gene product, had consistently been characterized by different laboratories as cation channels featuring significant but low calcium ion (Ca^{2+}) permeability (P_{Ca}/P_{Na} ranging from 1.1. to 5) (Estacion, Sinkins, Jones, Applegate, & Schilling, 2006; Kamouchi et al., 1999; Ko, Myeong, Yang, & So, 2017) already in the beginning of TRP research. Only TRPC1 emerged as an exception, as it was found to barely function as a cation channel when studied as a homomeric channel complex in overexpression systems. Despite the ample knowledge gained on regulatory and biophysical properties of recombinant TRPC channels, including delineation of complex lipid-mediated activation gating processes (Hofmann et al., 1999; Lichtenegger et al., 2018), the function of native TRPC channels remained largely elusive. Neither classical genetic approaches (gene knock-out and overexpression) nor

Abbreviations: ABCTC, Azo-BCTC; AC-4, Azo-Capsazepine 4; AzCAs, Azo-capsaicin derivatives; BCTC, 4-(3-Chloro-2-pyridinyl)-N-[4-(1,1-dimethylethyl)phenyl]-1-piperazinecarboxamide; Ca2, Calcium; CCh, Carbachol; cryo-EM, Cryo-electron microscopy; DAG, Diacylglycerol; 1,2-DOG, 1,2-di-O-octanoylglycerol; FFAzos, Photoswitchable fatty acid analogues; OptoDArG, Photoswitchable DAG with two arachidonic acid mimetic azobenzene moieties; PD, Parkinson's disease; PhoDAG, Photoswitchable DAG with one azobenzene moiety; PKC, Protein kinase C; PTL, Photochromic tethered ligand; TRP, Transient receptor potential; TRPC, Transient receptor potential canonical.

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https://doi.org/10.1016/j.pharmthera.2019.04.003

studies utilizing common experimental tools, which block cation channels in a rather non-specific manner, yielded a conceivable and consistent concept on the (patho)physiological role of mammalian TRPC proteins. Of note, TRPC channel proteins are prominently expressed in a wide range of cell types throughout the body, while a significant impact of TRPC conductances on tissue and organ functions was reported primarily in certain pathological/dysfunctional states in specific cell types such as cardiomyocytes, vascular smooth muscle cells, endothelial cells, podocytes and cerebellar Purkinje cells (Becker et al., 2009; Bush et al., 2006; Dietrich et al., 2005; Poteser et al., 2008; Winn et al., 2005). In line with this picture, the global knock-out of all seven TRPC isoforms (TRPC1-7 knock-out) in mice resulted in viable animals that lack severe malfunctions according to initial characterizations (Birnbaumer, 2015; Lutas, Birnbaumer, & Yellen, 2014). Importantly, investigations in model systems of pathological remodeling and in tissues with specific TRPC overexpression or channel dysfunction yielded compelling evidence for a linkage between TRPC conductances and diseased states including neurological, cardiovascular and kidney diseases (Tai, Yang, Liu, & Shao, 2017; Tiapko & Groschner, 2018). The inherent uncertainties in delineating the cellular functions of these signaling proteins by genetic approaches and/or use of barely specific inhibitors, generated a quest for better, TRPC isoform-specific, pharmacological tools. A particular challenge in TRPC and TRP research in general is the multimodal activation characteristics of these ion channels, embracing a wide range of physical as well as chemical stimuli (Lichtenegger & Groschner, 2014; Tiapko & Groschner, 2018). Physiological activation of TRPC channels typically involves input from G protein-coupled receptor pathways associated with the generation of lipid mediators such as diacylglycerols (DAGs), which trigger a number of non-TRPC-dependent, off-target, downstream events (Zhu, Jiang, & Birnbaumer, 1998). Therefore, conclusive evaluation of the cellular role of a TRPC conductance is difficult in a strictly physiological setting. Moreover, the features of channel complexes formed by a given TRPC isoform in distinct cell types or phenotypes are likely variable, giving rise to highly tissue and cell-type specific signaling patterns, which are considered as the basis of TRPC pathologies. Efforts have been undertaken to identify lead structures suitable for specific manipulation of TRPC channels (Beech, 2013; Hofmann, Schaefer, Schultz, & Gudermann, 2002). However, most of the earlier studies focused on recombinant homotetramers activated by divergent chemical stimuli or mixtures of activating agonists. Activation protocols were typically chosen to produce robust and persistent current activation. Thereby an array of chemical structures exhibiting inhibitory or activating potency at TRPC channels along with a certain degree of selectivity among the TRP family and the related superfamily of cation channels has been identified. Increasing awareness about cell-type specific oligomerization of TRPCs has dramatically improved the success in identifying compounds that target native, hetero-oligomeric TRPC channels, of a defined tissue-specific complex stoichiometry as exemplified for TRPC1/4/5 heteromers in the brain (Hofmann et al., 2002; Rubaiy et al., 2017; Storch, Forst, Philipp, Gudermann, & Mederos, 2012). Nonetheless, specificity and efficiency of pharmacological interference with disease-relevant TRPC signaling signatures is still poor and requires refined strategies and technology. Effective targeting and therapeutic manipulation of TRPC channels within a dysfunctional tissue might be achieved by drugs displaying high molecular selectivity as well as efficacy to interfere with TRPC function in the pathological setting, allowing for specific disruption of TRPC-mediated disease progression. However, tools that directly and specifically activate such native TRPC channels and, hence, enable to clarify their roles in pathophysiology, linkage to downstream signaling pathways and spatiotemporal aspects of pathological signaling are urgently needed to advance the field towards clinical applications.

Two recent achievements, the gain of structural information on TRPC complexes at the atomic resolution level and the advent of TRPC/lipid photopharmacology are considered to provide the awaited boost for this development. Our understanding of the molecular basis of TRPC pharmacology has benefitted remarkably from the very recent insight into the 3D structure of these channels gained by single-particle cryo-electron microscopy (cryo-EM) studies (Azumaya, Sierra-Valdez, Cordero-Morales, & Nakagawa, 2018; Duan et al., 2018; Fan, Choi, Sun, Du, & Lu, 2018; Sierra-Valdez, Azumaya, Romero, Nakagawa, & Cordero-Morales, 2018; Tang et al., 2018; Vinayagam et al., 2018). A recent series of reports provided high (3.3–4.4 Å)-resolution models of homomultimeric TRPC complexes. These investigations covered so far three individual TRPC isoforms (TRPC3, 4 and 6) and included also the structures of certain lipid- and ion-occupied channel states. All these structures displayed a restricted permeation pathway, thus representing non-conducting, closed (resting) or inactivated states (Fan et al., 2018; Tang et al., 2018). This scenario was particularly unexpected for channels reconstituted in nanodiscs containing a diacylglycerol, known to act as a potent lipid activator of the channel. Of note, some of the TRPC structures resolved as yet were clearly demonstrated to harbor multiple, regulatory lipid mediators. One conclusion from this finding is that TRPC channels are likely to undergo multiple, time-dependent gating processes, including current- and/or ligand-dependent channel inactivation. Hence, pharmacological inhibition of TRPC signaling may be conferred not only by blocking the permeation pathway but also by locking the channel into an inactivated state, which is typically obtained by channel agonists. Moreover, this concept highlights the importance of temporal signaling aspects with respect to both channel pathophysiology as well as pharmacology. Drug sensitivity of TRPC channels in a target tissue appears to depend on structural and spatial aspects such as subunit stoichiometry or the channels´ cellular targeting within the cell. Structural features, like dynamic interaction with certain cytoskeleton components were recently recognized as determinants of functional states that confer sensory features and enable channel activation by lipid mediators via a temporal pattern of activating stimuli (Storch et al., 2017). Lipid-mediated activation gating of TRPC channels has emerged as the pivotal mechanism in physiological and pathophysiological TRPC signaling. Methods for direct and precise control of these processes are required to decipher their exact role in pathophysiology of certain tissues and organs, and to enable effective screening for suitable inhibitors. A respective breakthrough was achieved by recent developments in lipid photopharmacology, which enabled the photo-reversible manipulation of TRPC channels directly, bypassing upstream signaling pathways, with the spatial and temporal precision with which light can be applied. Application of photochromic lipid mediators provided unprecedented insight into the molecular sensing machinery of TRPC channels. A study combined “optical lipid clamp” technology with a structure-guided mutagenesis approach and identified a TRPC3 domain involved in lipid-gating and lipid recognition (Lichtenegger et al., 2018). These results were consistent with the localization of lipid densities within the TRPC3 channel by single-particle cryo-EM (Fan et al., 2018).

Novel concepts for high-precision manipulation of TRPC channels together with the increasing structural knowledge on the molecular action of modulators and blockers of TRPC channels are expected to unravel and define pathological TRPC signaling patterns, and pave the way towards their therapeutic manipulation. Light-mediated manipulation of TRPC signaling will not only help to better understand these channels as therapeutic targets and to develop efficient drug screening platforms, but also to advance photopharmacological concepts towards clinical applications. In the following, we aim to provide a step by step introduction into these novel therapeutic aspects, starting with a basic introduction to TRP channels as a target of photopharmacology.

2. TRP channels as a target of photopharmacology

2.1. A brief introduction to ion channel photopharmacology

Classical pharmacological strategies for the investigation of signaling proteins including ion channels are typically limited by vague selectivity

and poor control over concentrations at the biological target sites. The concept of photopharmacology appears to provide a solution to these problems and is developing fast in recent years. This technique employs light as an external trigger to unleash biological activity of the ligands or even for ultimately precise reversible control of the activity of agonists, channel openers or blockers. A variety of photoconvertible molecules comprise the toolbox for development of light-controlled ligands that bind to and modulate signaling mediators. There are three general, fundamentally different photopharmacological strategies used, which are based on caged ligands, photochromic ligands and photochromic tethered ligands (PTL) (Fehrentz, Schönberger, & Trauner, 2011). Caged biological agents consist of an activity-preventive, light-sensitive group, typically designated as cage, which is attached to the ligand via covalent bonding. Light irradiation, absorbed by the cage, eliminates the protective bond and releases the ligand. The most frequently used caging groups are nitrobenzyl and coumarin moieties. Caged ligands like neurotransmitters, adenosine triphosphate as well as regulatory ions, in particular Ca^{2+}, have been successfully used in neurophysiological investigations (Housley, Raybould, & Thorne, 1998; Kötter, Schubert, Dyhrfjeld-Johnsen, Luhmann, & Staiger, 2005; Rial Verde, Zayat, Etchenique, & Yuste, 2008; Wang et al., 2013). With this strategy a certain level of the caged precursor is applied within a tissue, cell or an intracellular compartment without activating the biological system or initiating feedback loops or metabolic degradation. This sets the stage to activate the target with high spatiotemporal precision by uncaging the biologically active entity. Caged ligands are, however non-reversibly light-controlled tools and therefore do not allow for cyclic signal activation, and uncaging processes were shown to be associated with biological side effects (Il'ichev, Schwörer, & Wirz, 2004; Tiapko et al., 2016). Deactivation cannot be controlled, and is ill-defined depending on degradation and metabolism as well as removal of the active ligand from its target site by various transport processes. These disadvantages are conceptually overcome by the use of reversibly photoswitchable ligands (photochromic ligands).

The photochromic ligand strategy enables introduction of fully reversible, light-controlled biological activity. This is achieved by incorporation of a flexible photoisomerisable moiety into the structure of the ligand. Light-mediated transition between *cis*- and *trans*-conformation in the photochromic moiety is induced by irradiation at distinct wavelength according to the absorption spectra. Relaxation from *cis*- to the thermodynamically more stable *trans*-state occurs also in the absence of light. As yet azobenzene has been established as one of the most suitable and reliable light-sensing moieties. Two main advantages of this photoswitch are: azobenzene absorbs even at relatively low intensities of light and isomerization is essentially fast. Apart from the essentially fast single-molecule photoswitching kinetics (picoseconds range), azobenzenes are characterized by fast and efficient kinetics also at the molecule population level, which is prerequisite for biological application. Azobenzene absorption spectra of *cis* and *trans* form are well separated, with sufficiently high extinction coefficients and quantum yields to enable fast and highly efficient cycling between photochemical states featuring more than 90% of the azobenzene molecules in alternating conformations. (Kobayashi & Saito, 2002; Samanta, Qin, Lough, & Woolley, 2012; Siewertsen et al., 2009). Of note, the fact that bulk kinetic features of photoswitches may vary with biological environments needs consideration. Nonetheless, azobenzene-based photochromic ligands can be used for accurate monitoring of association-disassociation kinetics between the photoswitchable ligand and its target protein (Fehrentz et al., 2011). Spatiotemporally precise control over ion channels by azobenzene-containing ligands was successfully employed to investigate voltage- and ligand-sensitive proteins including voltage-gated potassium ion channels, voltage-gated sodium ion channels, as well as TRPs channels (Hüll, Morstein, & Trauner, 2018). Of note, spatial precision of the pharmacological intervention with photochromic ligands is limited not only by the diffraction limit but also by diffusion of the active species outside the irradiated volume element, and specificity for the target is frequently insufficient as in conventional pharmacology.

To obtain ultimate target specificity and also spatial resolution, the photoswitchable ligand can be covalently linked to its target molecule in a strategy termed PTL. This strategy typically requires high-resolution structure information on the target as well as genetic interventions and cross-linking chemistry suitable for live cell investigations. Typically, by genetic engineering cysteine residues are incorporated in close proximity to the ligand binding site, and a maleimide group is required within the photoswitchable ligand to couple to the cysteine residue and enable functionally suitable attachment of the bioagent. This technology enables not only reversible light-controlled ligand binding but also excludes off-targets. The tethered ligand approach has been proven highly reliable and efficient in studies on voltage- and ligand-gated channels (Banghart, Borges, Isacoff, Trauner, & Kramer, 2004; Fortin et al., 2011).

Chemogenetics provides an alternative avenue to enhance specificity for a desired target. Herein a genetic intervention creates artificial sensitivity of the target protein to specific bioagents (activators or blockers). Chemogenetics can be combined with photopharmacology not only in terms of the PTL approach (Fehrentz et al., 2011), but also as a strategy to further enhance specificity and efficacy of photochromic ligands. The introduction or modulation of ligand sensitivity in ion channels is an attractive technology to overcome the problems with off-target effects and low potency or efficacy of available photoswitchable ligands (Sternson & Roth, 2014). Genetic manipulation of TRPC channels to generate supersensitivity towards a designer drug has recently been reported for TRPC3 (Svobodova et al., 2019).

2.2. Current molecular tools for optical control of TRP channels

TRP channels serve as signal integrators in diverse tissues and are able to sense multiple input modalities, which govern channel gating (Clapham, 2003). Lipid composition and the distribution of lipid species within the plasma membrane change in response to the activation of plasma membrane receptors and during metabolic and morphological adaptation of the cell (Sunshine & Iruela-Arispe, 2017). Certain membrane lipids, which function to report changes in the cell environment and its metabolic or functional state, are recognized by TRP channel complexes. The information generated by lipid mediators and specific components of the lipid bilayer is efficiently transduced by TRP channels into plasma membrane ion fluxes (Ciardo & Ferrer-Montiel, 2017). Consequently, the first attempts to control the activity of TRP channels by photopharmacology were based on lipids and lipophilic channel activators including DAGs, which represent endogenous regulators of various TRPC channel subtypes, such as TRPC3 and TRPC6 (Hofmann et al., 1999). Early studies adopted the caged ligand strategy width DAG derivatives featuring a coumarin- or nitrobenzyl-derived caging moiety to mask agonist function of the lipid and prevent its biological activity (Fig. 1A). Illumination with light of appropriate wavelength cleaves the caged ligand and quickly releases the active lipid along with the caging structure that is considered as biologically fairly inert. The first caged DAG was introduced back in 1996 through nitrobenzyl caging of the DAG hydroxyl group, which is a moiety responsible for the most of DAG interactions with cellular proteins (Höglinger, Nadler, & Schultz, 2014; Huang, Sreekumar, Patel, & Walker, 1996). Importantly, coumarin- and nitrobenzyl-caged lipids differ in their mechanisms and photophysical properties of uncaging. In this respect, coumarin-derived cages have several advantages over nitrobenzyl derivatives. Both nitrobenzyl and coumarin groups are photoactivated with wavelengths in the UV range, which is not suitable for biological application due to cytotoxicity. Nitrobenzyl-derivatives have absorption maxima around 300–350 nm, while some of the coumarin-derivatives can be photoactivated with slightly higher wavelengths, such as within visible range (around 400 nm), which could make the uncaging process less cytotoxic. However, the more important

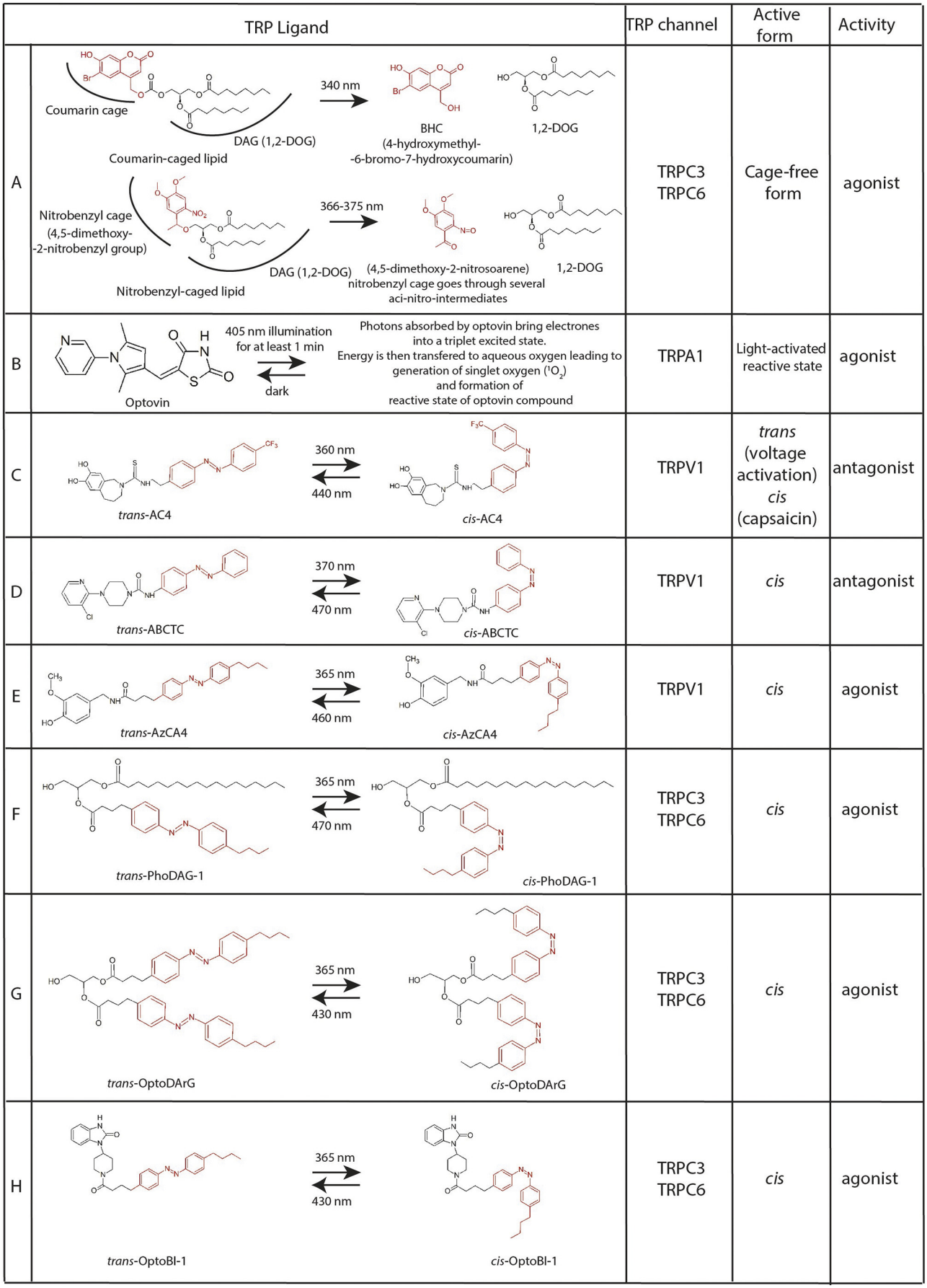

Fig. 1. Chemical structures of photochromic TRP actuators.

advantage of coumarin-derived caging groups is that the photoreaction is characterized by higher extinction coefficients and higher quantum yields, which results in requirement of lower light intensities for uncaging. Due to the efficient, relatively simple uncaging reaction, release of coumarin-derived cages is rapid in comparison to nitrobenzyl groups and occurs within nanoseconds, whereas the cleavage of nitrobenzyl cages occurs through several nitro-intermediates (Höglinger et al., 2014). Therefore, coumarin-derived caged lipids were considered more appropriate to investigate the relatively rapid signaling processes triggered by DAGs that typically occur within seconds or minutes. Another aspect important for interpretation of results obtained with these photopharmacological tools is the chemical structure of the active lipid species. The first caged DAGs included short chain DAG analogs such as 1,2-di-O-octanoylglycerol (1,2-DOG), which is not a naturally occurring lipid mediator. Nonetheless, application of caged 1,2-DOG was found suitable to investigate a series of cellular signaling pathways, including DAG-mediated reorientation of microtubule-organizing center in primary T-helper cells (Quann, Merino, Furuta, & Huse, 2009), myocyte contractility and PKC activity (Huang et al., 1996) as well as TRPC3-mediated Ca^{2+} signaling (Tiapko et al., 2016). However, there is substantial diversity of endogenous DAG species, which are considered to have different biological properties (Eichmann & Lass, 2015). Indeed, recent investigations with caged DAGs, which resemble more closely the endogenous mediators, showed that the structure of acyl chains has substantial impact on activity. Caged stearoyl-arachidonyl glycerol was the most potent activator of TRPC3- and TRPC6-mediated Ca^{2+} entry and PKCα translocation to the plasma membrane in HeLa cells (Nadler et al., 2013). Although the development of caged DAGs was an important step towards control of TRP channels by light, this method suffers from a few shortcomings. Besides the above mentioned lack of reversibility of the activation process, caging structures released during activation were found to exert significant biological effects and interfere with downstream signaling (Il'ichev et al., 2004; Tiapko et al., 2016).

Reversible and cyclic optical control of a TRP channel was first exemplified by the use of optovin, a small molecule TRPA1 photochromic ligand (Fig. 1B) (Kokel et al., 2013). Optovin was found suitable for repetitive activation of human TRPA1 upon violet light illumination in HEK293 cells overexpressing TRPA1 as well as native murine TRPA1 channels in dorsal root ganglion sensory neurons. Moreover, optovin was also effective *in vivo* in zebrafish where it enabled optical control of zebrafish dorsal fin movements. The mechanism proposed for optovin-mediated control of TRPA1 is the reversible formation of a covalent thioether bond between a light-induced optovin intermediate and redox-sensitive cysteine residues in the TRPA1 channel at positions 621, 633 and 856 (Fig. 2A). Interestingly, this activation was rapidly reversed in the dark, although the exact deactivation mechanism is not known (Kokel et al., 2013). The same group later showed that optovin-mediated TRPA1b activation can also be used to *in vivo* control zebrafish heart rate and pacing of human stem cell-derived cardiomyocytes (Lam et al., 2017).

An important next step was the introduction of azobenzene-based photoswitchable TRPV1 agonists/antagonists by Dirk Trauner's group. By inclusion of azobenzene moieties into the structures of the TRPV1 antagonists capsazepine and 4-(3-Chloro-2-pyridinyl)-N-[4-(1,1-dimethylethyl)phenyl]-1-piperazinecarboxamide (BCTC), photoswitchable TRPV1 antagonists Azo-Capsazepine 4 (AC-4) and Azo-BCTC (ABCTC) were generated (Fig. 1C and D) (Stein, Breit, Fehrentz, Gudermann, & Trauner, 2013). *Trans* AC-4 was found to function as antagonist of TRPV1, when activated by voltage, whereas in this setting ABCTC acted as antagonist in its *cis* conformation. Interestingly, for capsaicin-activated TRPV1, AC-4 was characterized as a *cis* antagonist, while ABCTC was unable to inhibit capsaicin-induced TRPV1 currents. This demonstrates the high complexity of interaction between photochromic ligands and their targets, which may even expose divergent receptor domains depending on the presence of other modulatory stimuli. Cryo-EM of TRPV1 complexes incorporating the vanilloid agonists resiniferatoxin or capsaicin identified the vanilloid binding pocket within TRPV1 (Cao, Liao, Cheng, & Julius, 2013). Residues lining the vanilloid pocket of TRPV1 essential for capsaicin binding are Y511 in S3, T550 and M547 in S4, E570 in S4-S5 linker and L669 in S6 of the neighboring subunit. Capsazepine and AC-4 binding sites are most probably located within this pocket (see Fig. 2B). It is tempting to speculate that ABCTC binds to this capsaicin binding domain, as pharmacological experiments indicated that capsazepine and BCTC compete for the same binding site (Gavva et al., 2005).

Based on the structure of capsaicin, photo-switchable TRPV1 agonists (AzCAs) were developed, among which AzCA4 was most effective (Fig. 1E). The AzCA4 structure is comprised of the capsaicin head group and an azobenzene-containing photoswitchable fatty acid tail (Frank et al., 2015). Again structural similarities between AzCA4 and vanilloid TRPV1 agonists, along with the fact that capsazepine completely abolished AzCA4 activity, led to the conclusion that AzCA4 binds at the vanilloid binding site within TRPV1 channel (Fig. 2B).

A paradigm of lipid gating in TRP channels is the activation of TRPC channels by DAG. Experiments using DAGs to investigate the function of native TRPC channels and their gating principles have so far failed to produce significant advances. These hurdles were overcome when photochromic ligands based on azobenzene moieties have subsequently been generated for TRPC channels. Initially introduced by Dirk Trauner as PhoDAGs, they featured photoswitchable fatty acid analogues (FFAzos) within the DAG structure (Frank et al., 2016) (Fig. 1F). PhoDAG-1 is chemically related to 1,2-stearoyl-arachidonyl glycerol, whereas PhoDAG-2 and PhoDAG-3 most likely mimic 1,2-DOG. PhoDAGs enabled rapid and spatially precise investigation of various DAG signaling pathways, including not only TRPC conductances, but also recruitment and translocation of C1 domain-containing proteins and PKCδ to the plasma membrane (Frank et al., 2016). PhoDAGs were demonstrated suitable not only for the control of recombinant TRPC channels, but also endogenously expressed, native TRPC channels in HeLa cells, mouse vomeronasal sensory neurons and sensory neurons of olfactory epithelium as well as vomeronasal organ tissue slices (Frank et al., 2016; Leinders-Zufall et al., 2018). More recently, a modified photoswitchable DAG structure (OptoDArG; Fig. 1G) (Lichtenegger et al., 2018), which contains two arachidonic acid mimetic azobenzene side chains, was identified as a highly efficient photochromic activator of TRPC3 channels. Of note, this photoswitchable lipid outperforms PhoDAGs in terms of TRPC channel activation specifically for TRPC3 and, interestingly, gains efficacy by a point mutation in TRPC3 (G652A), which eliminates sensitivity to endogenous activation pathways (Lichtenegger et al., 2018). This phenomenon opens the view on chemogenetic manipulations that may increase specificity as well as efficiency of TRPC photopharmacology as further discussed below.

Furthermore, recently we introduced azobenzene into the structure of TRPC3/6 benzimidazole agonist GSK1702934A to developed the first small molecule photoswitch for the control TRPC3/6 activity. This compound, known as OptoBI-1 (Fig. 1H) enables lipid-independent activation of TRPC3 channel in both HEK293 heterologous expression system and in native tissues such as endothelial cells and hippocampal neurons (Tiapko et al., 2019).

In a nutshell, azobenzene photoswitches enable in-depth mechanistic studies of TRP channel function, including activation, inactivation and deactivation mechanisms as well as downstream signaling processes. Exploiting the temporal precision of TRPC3 gating by OptoDArG, we were able to identify a conserved residue in the TRPC pore domain (G652 in TRPC3 and G709 in TRPC6), which is crucial for primary structural recognition/discrimination of DAG species by the channel complex (Fig. 2C and D) (Lichtenegger et al., 2018). Photochromic DAGs enabled the first reasonable analysis of the channel/DAG interaction kinetics and the characterization of TRPC channels within distinct subcellular localizations (Leinders-Zufall et al., 2018), based on the virtually

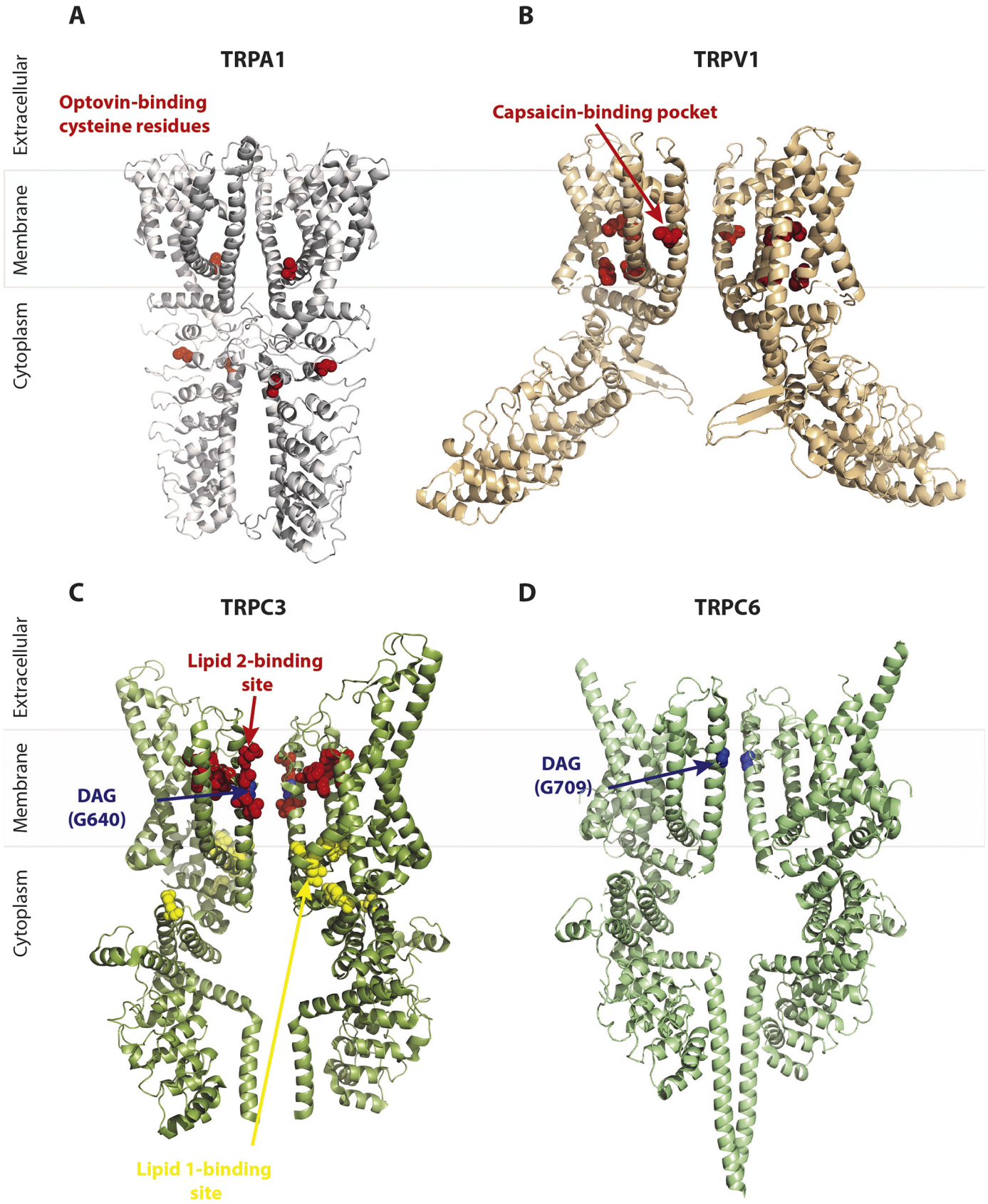

Fig. 2. 3-D models of TRPs with photochromic ligand-binding sites. (A) Structure of TRPA1 channel. Cysteine residues which are proposed for optovin-binding are shown in red (C621, C633 and C856; (Kokel et al., 2013)). (B) Structure of TRPV1 channel with its capsaicin-binding pocket. Residues essential for capsaicin binding are Y511 in S3, T550 and M547 in S4, E570 in S4-S5 linker and L669 in S6 of the neighboring subunit (Cao et al., 2013). (C) Structure of human TRPC3 channel with two lipid densities as identified by cryo-EM (lipid 1 shown in yellow, lipid 2 shown in red; (Fan et al., 2018)). Residues important for the interaction with lipid 1 within the pre-S1 elbow, S1, and the S4-S5 linker are K241, Y323, I544, V341, K337, I544, N548, F551. The second lipid density resides between S6 and P loop of adjacent subunits. (D) Structure of the TRPC6 channel (Tang et al., 2018) with marked (blue) residue G709 (Lichtenegger et al., 2018), which is an equivalent residue to G652 in TRPC3. PDB codes: TRPA1 (3J9P), TRPV1 (3J5P), TRPC3 (6CUD), TRPC6 (5YX9).

instantaneous transition between active and inactive lipid conformations and the spatial resolution of light-induced activation.

At this point, it is important to note that so far photochromic tethered ligands(PTL) or optical gating strategies with bivalent, crosslinking photoswitches have not been established for TRPC channels.

2.3. From photochromic lipids towards opto-chemical genetics of TRPC channels

Lipid photopharmacology has proven valuable for mechanistic studies, especially for the analysis of structure-function relations and for evaluation of the role of specific cells and cellular structures in lipid signaling (Frank et al., 2015; Leinders-Zufall et al., 2018; Lichtenegger et al., 2018). However, photochromic DAGs are essentially nonspecific channel actuators as they affect a diverse array of signaling pathways. The lack of specificity argues against suitability of these optical TRPC actuators for development of new therapeutic concepts, even considering the spatiotemporal precision of photopharmacology. Nonetheless, our recent gain in understanding of ligand recognition in TRPC channels (Lichtenegger et al., 2018) and the steadily growing repertoire of synthetic channel activators and blockers (Tiapko & Groschner, 2018) open the view on sufficiently specific and precise interventions that might be developed into therapeutic strategies. Both lipid components of the cell plasma membrane including DAGs as well as synthetic ligands have been localized within the channel complex structure, indicating the molecular position of ligand binding domains in TRPC channels (Fan et al., 2018; Tang et al., 2018). One interesting aspect arises from the finding that chemical sensitivity in TRPC channels can be efficiently engineered. Genetic introduction of high sensitivity towards an unnatural lipid mediator (OptoDArG) has recently been shown as the consequence of a single point mutation in TRPC3 and TRPC6 (Figs. 2C–D, 3A–D) (Lichtenegger et al., 2018). Importantly, the same point mutation blunts the channel's responsiveness to its endogenous activators and preserves or even enhances the efficacy of benzimidazole agonists such as GSK. Hence, TRPC proteins with genetically modified chemosensitivity might enable high-specificity activation of TRPC-linked downstream signaling pathways. This is in particular intriguing in view of the possibility of dominant transfer of genetically introduced features to native channel complexes upon heteromerization, as already exemplified for other pore-domain mutations (Poteser et al., 2006, 2011) (Fig. 3E–F; unpublished observation). Such genetic engineering of the ligand sensitivity of endogenous TRPC channels may well be combined with the high spatiotemporal precision of optical actuation by certain photochromic lipids or novel photoswitchable synthetic activators such as benzimidazoles. The therapeutic value of high-precision targeting of TRPC signaling processes by light needs careful evaluation. In general, the most obvious challenge to advance such strategies is the development of actuators that cycle in their conformational states by absorption of red-shifted light or new technologies for utilization of red light actuation of conventional photoswitches, to enable the required penetration depth into a target tissue as already reported for immunotherapeutic targeting of skin cancer (He et al., 2015a). There are indeed several potential applications that might be developed from TRPC photopharmacology and opto-chemical genetics. An account of the current therapeutic relevance of TRPC pharmacology is given below.

3. The therapeutic potential and limitations of high precision optical manipulation of TRPC function

3.1. Opportunities and hurdles

TRP channels govern a wide range of physiological/pathophysiological processes and their role in human diseases is being increasingly recognized. Consequently, these ion channels gain steadily interest as therapeutic targets. However, most agents that activate/inhibit TRP channels are expected to substantially interfere with essential and ubiquitous signaling pathways. The advent of TRP photopharmacology opened the view on a novel, noninvasive and spatiotemporally precise approach to manipulate TRPC function (Frank et al., 2016; Lichtenegger et al., 2018). It is expected that more and more tools for the optical interference with TRPC signaling will become available soon. Such molecules and strategies might be of therapeutic value when the target cells are accessible to light irradiation. Temporal precision itself, but even more importantly, the unique option of temporal control over the cycling of channels between distinct functional states (resting, activated, inactivated, desensitized) offers the view on an exceptionally efficient and specific intervention. As an example, cycling of TRPC3 channels between activated and deactivated states by a photochromic ligand largely bypasses the desensitization phenomenon that is typically observed with conventional channel activators (Lichtenegger et al., 2018). Moreover, a novel photochromic modulator of TRPC channels (OptoBI-1) appears suitable for controlled introduction of distinct TRPC activity levels, which include a constitutively active state and a fully closed state (Tiapko et al., 2019). Therefore, photopharmacology offers not only a spatial restriction of the intervention but also a pharmacological interference that qualitatively and quantitatively reaches beyond that of conventional pharmacology with blockers or activators. The therapeutic value of these manipulations, as well as the suitability to obtain a high tissue and cell-type specificity, needs now careful evaluation. The spatial precision of photopharmacology may enable the safe and efficient use of systemically toxic drugs, while application of a particular temporal pattern of channel activity may allow for tissue-specific interference including also the control over excitable cells and linked organ functions.

A major limitation of the photopharmacological approach is given by the required accessibility to light. In this respect issues have been classified into five groups. For interventions at the body surface (skin, eye), completely noninvasive therapy is enabled (class one organs). The second and third group comprise organs, which can be easily reached by endoscopy (gastrointestinal tract, bladder, cervix, respiratory system and biliary system) or reside in surface proximity just under the skin (thyroid, lymph nodes, muscles and some bones) (Lerch, Hansen, van Dam, Szymanski, & Feringa, 2016). Importantly, UV light, which is typically used to activate azobenzene photoswitches, penetrates only a few mm. In addition, it is potentially harmful and bears the risk of irradiation side effects (Lee, Wu, Hong, Yu, & Wei, 2013). Hence, shifting the excitation maximum of photoswitches towards higher wavelengths, which are less damaging and feature higher penetration depth, is the main challenge and prerequisite for the successful development of therapeutic concepts. The near-infrared range between 650 nm and 900 nm is the most suitable, since light of lower wavelengths is absorbed by endogenous chromophores, mostly hemoglobin, and higher than 900 nm by water, which represents the limitation (Weissleder & Ntziachristos, 2003). Importantly, a new class of tetra-ortho-substituted azobenzenes have been developed, whose trans-cis isomerization is induced by visible light (Beharry, Sadovski, & Woolley, 2011; Samanta, McCormick, Schmidt, Seferos, & Woolley, 2013), which renders them less toxic and enables deeper penetration. However, when considering manipulation of inner organs, penetration depth of wavelengths in the near-infrared range is hardly sufficient without application of new optical technologies. For example, light of 800 nm can penetrate around 2 cm (Kalka, Merk, & Mukhtar, 2000; Velema, Szymanski, & Feringa, 2014). Hence, in the case of the inner organs, which have been classified into the groups four (such as pancreas, liver, kidneys, intestine) and five (brain and bone marrow) (Lerch et al., 2016), the application of photopharmacology would require minor or major incisions and operations. Here, a big advance represents the replacement of fiber-optic light sources with wireless implantable LED devices, as small as a few millimeters, that can be implanted into the body to locally activate

the drug (Hüll et al., 2018; Montgomery et al., 2015; Park et al., 2015). Moreover, new illumination advance in wireless optogenetics are near-infrared-light activatable nanoparticle. They enable application of deep-tissue penetrating near-infrared light which is then within lanthanide-based nanoparticles converted into visible light to activate its target (He et al., 2015b).

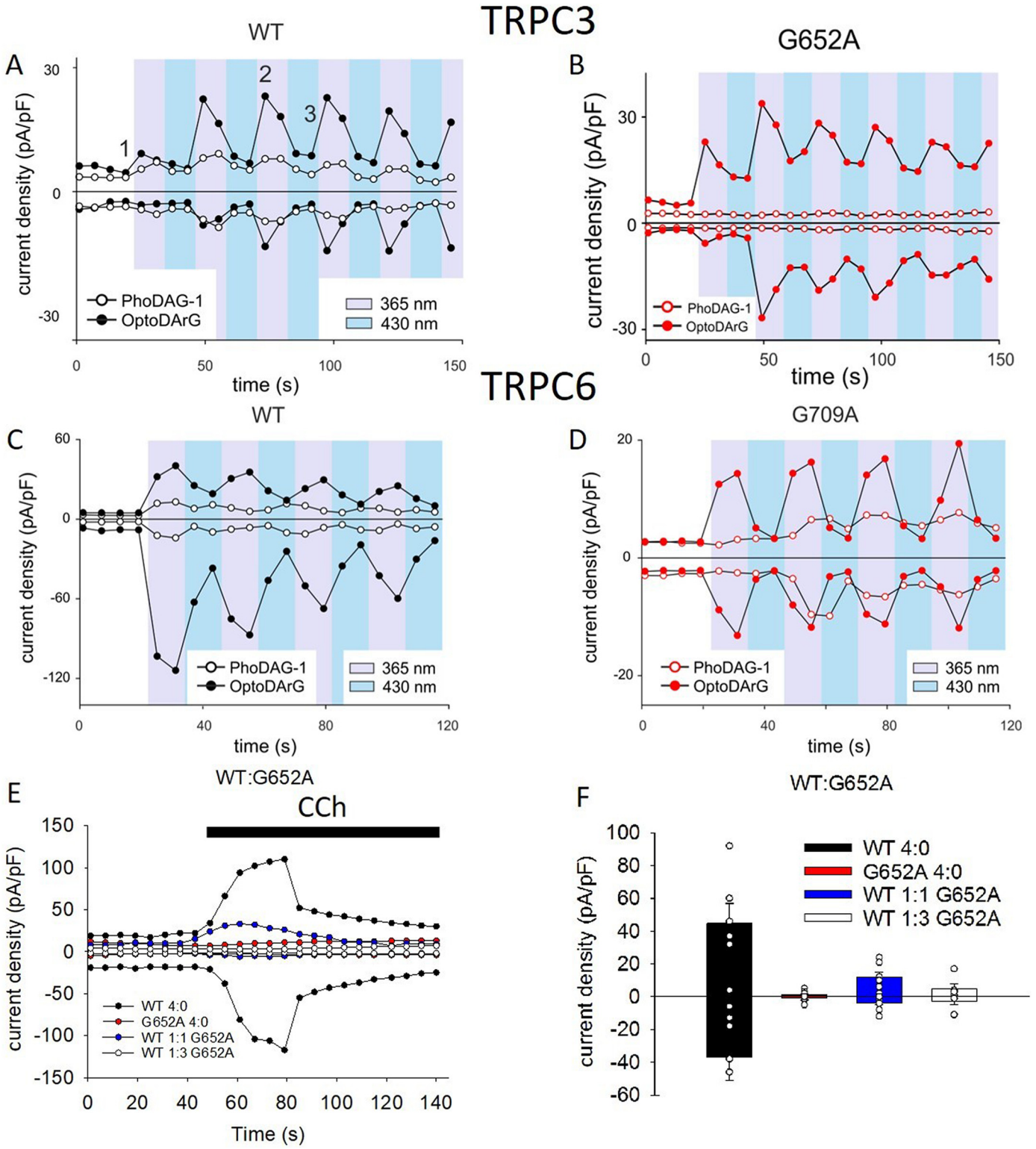

Fig. 3. Photopharmacological and chemogenetic manipulation of TRPC channels. TRPC3 WT (A) and G652A mutant (B), TRPC6 WT (C) and G709A mutant (D) representative time courses of current development during repetitive photoconversion of OptoDArG and PhoDAG-1. Illumination with UV (365 nm) and blue (430 nm) light was switched every 10 s of the recording. ((A), (B) and (C) are reproduced from (Lichtenegger et al., 2018) with permission of publisher Nature Chemical Biology, Springer Nature Limited, 2018 (https://creativecommons.org/licenses/by/4.0/)). Unpublished observation: E) Dominant-negative suppression of carbachol (CCh)-induced (100 μM; indicated) response of TRPC3 WT in presence of G652A mutant channels. Representative time courses of current development recorded in HEK cells with different co-expression ratio (only TRPC3 WT (black; 4:0) or G562A (red; 4:0); co-expressed TRPC3 WT and G562A (blue; 1:1 and white; 1:3 respectively). F) Current densities (at −90 mV and + 70 mV) elicited by CCh application to HEK cells co-transfected with varied TRPC3 and G652A mutant channels ratio (as indicated). Data are shown as mean ± SEM.

Apart from the above outlined illumination and tissue localization issues, pharmacodynamic and pharmacokinetic properties of the photochromic molecules need careful consideration. Similar to classical pharmacology, absorption, distribution, metabolism and excretion are crucial for therapeutic feasibility. The ideal scenario is that the photochromic molecule, in its inactive form, can be administered orally to reach its target where it is converted into the active isomer by local illumination. Optovin has successfully been applied *in vivo* in zebrafish (systemically) and in mice (locally as swab on an ear) for the control of TRPA1 channel, without notable toxicity reported (Kokel et al., 2013). Photoswitchable capsaicin analogue AzCA4 has been used to activate TRPV1 channel *ex vivo* in murine dorsal root ganglion neurons and C-fiber nociceptors (Frank et al., 2015). Although currently available photopharmacological activators for the control of TRPC3/6 channels have been successfully applied on primary cells (Leinders-Zufall et al., 2018; Lichtenegger et al., 2018; Tiapko et al., 2019), *in vivo* data about their pharmacokinetic features are as yet lacking.

DAG-based agonists, such as PhoDAGs and OptoDArG, are known to be nonselective and to activate various other targets in the cell (Frank et al., 2016; Leinders-Zufall et al., 2018; Lichtenegger et al., 2018). Therefore, small molecule TRPC activators/inhibitors, which use lipid-independent pathways to modulate TRPC activity, might be more appropriate. The recently published photoswitchable TRPC3/6 agonist OptoBI-1 is an azobenzene derivative of benzimidazole compound GSK1702934A (Tiapko et al., 2019). Importantly, the lead compound GSK1702934A is reportedly selective for TRPC3/6 channels over other channels including TRPV4, TRPA1, M1, M4, CaV1.2, hERG, NaV1.5, or CXCR5 receptor (Wajdner et al., 2017; Xu et al., 2013). Currently available synthetic TRPC3/6 inhibitors are pyrazole and anilino-thiazole derivatives (Tiapko & Groschner, 2018). While the most extensively characterized pyrazole 3 appears to inhibit not only TRPC3, but also the STIM/Orai pathway, pyrazole 10 was found more selective (Kiyonaka et al., 2009; Schleifer et al., 2012). Furthermore, anilino-thiazoles GSK2332255B and GSK2833503A are potent and selective TRPC3/6 inhibitors (Washburn et al., 2013). However, they do not appear as suitable for *in vivo* application due to limited bioavailability. In addition, a study in an animal model of cardiac hypertrophy showed their low efficacy due to fast metabolism and a high level of protein binding (Seo et al., 2014). Most recently, a bioavailable and specific TRPC6 inhibitor DS88790512 has been reported (Motoyama et al., 2018). Photoswitches acting on TRPC1, TRPC4 and TRPC5 channels have not been reported yet. TRPC1 is typically found in heteromeric complexes with other TRPC isoforms and specific TRPC1 activators/inhibitors are as yet lacking. By contrast, for TRPC4/TRPC5 channels a range of small molecule inhibitors, which might serve as a useful basis for the synthesis of TRPC4/TRPC5 photoswitches, are available. Among them, ML204 (Miller et al., 2011) and Pico145 (Rubaiy, Ludlow, Bon, & Beech, 2017) are highly selective for TRPC4/5 and appear suitable for oral administration.

Incorporation of azobenzene into the active molecules potentially modifies pharmacodynamics as well as pharmacokinetics. For example, azobenzenes differ in their polarity and water solubility in *trans* and *cis* form (Brown et al., 2017). In addition, azobenzenes are sensitive to microsomal reduction (Zbaida, 1995) and also can easily be reduced by thiols, such as glutathione, especially in their *cis* form (Boulègue, Löweneck, Renner, & Moroder, 2007). This could pose a problem for *in vivo* application. However, careful design of the substitution pattern in the azobenzene moiety may overcome this problem (Samanta et al., 2013).

Further potentially limiting aspects are the stability of the active form and how the photoswitch will be inactivated and converted back into the inactive state. It is essential to consider how the isomers are metabolized/excreted as well as toxicity of metabolites. Interestingly, some azobenzene-based compounds such as sulfasalazine have already been successfully used in therapies (McGirt, Vasagar, Gober, Saini, & Beck, 2006).

3.2. Potential future applications

Therapeutic targeting of TRP channels is considered particularly promising for the control of pain. This therapeutic concept is based on a well-established role of TRPA and TRPV channels in pain sensation and has attracted substantial interest with respect to the treatment of pathological pain syndromes such as neuropathic pain, inflammatory pain and visceral pain, as has already been extensively reviewed (Dai, 2016; Jardin et al., 2017). The first steps towards the use of photoswitches in pain therapy are the development of the optical actuators of TRPA1 and TRPV1 channels (Kokel et al., 2013; Stein et al., 2013). The role of TRPC channels in pain sensation and detection is incompletely characterized. Nonetheless, TRPCs were reported to act in concert with other TRP channels in pain sensation. TRPC1, TRPC4, TRPC5 and TRPC6 are reportedly expressed in skin sensory neurons involved in cutaneous sensory functions as well as in dorsal root ganglion neurons contributing to chronic pain (Alkhani et al., 2014; Qu et al., 2012). TRPC3 together with TRPA1 appears involved in histamine-independent pain and itch (Than, Li, Hasan, & Zhang, 2013), whereas TRPC1 and TRPC6 interact with TRPV4 to mediate mechanic hyperalgesia associated with inflammation (Alessandri-Haber, Dina, Chen, & Levine, 2009). Interestingly, a recent study in a mouse TRPC5 knockout model, as well as inhibition of TRPC5 by ML-204, suggested that TRPC5 activation might be beneficial in amelioration of arthritis hyperalgesia and inflammation (Alawi et al., 2017). These observations indicate a potential value of targeting TRPC channels, along with other TRP channels, in the treatment of chronic pain. So far, clinical trials with TRPV1 antagonists uncovered severe side effects, such as hyperthermia, as well as impaired detection of heat pain (Dai, 2016). Hence, TRPV1 photopharmacology appears promising to prevent adverse effects by spatially restricted intervention.

The primarily most accessible and light-exposed organ is the skin. Expression of TRPC channels has been reported in several cell types important for skin function such as mucosal and epidermal keratinocytes, where they are involved in keratinocyte proliferation and differentiation and, hence, barrier formation (Beck et al., 2008; Cai et al., 2006; Müller et al., 2008). TRPC channels have been related to skin pathologies, such as psoriasis and skin fibrosis. On the other hand, in psoriasis and atopic dermatitis, which are characterized by disturbed differentiation and increased proliferation of keratinocytes, TRPC agonists/activators might be beneficial. In line with this concept, hyperforin, a TRPC6 activator, indeed was shown to induce differentiation of human primary keratinocytes and to inhibit proliferation (Müller et al., 2008). Furthermore, reduced expression of TRPC1, TRPC4 and TRPC6 channels in human psoriatic skin lesions was found associated with impaired Ca^{2+} homeostasis and keratinocyte dysfunction (Leuner et al., 2011). A beneficial role for TRPC channels was also suggested in wound healing. TRPC6 was found essential for fibroblast differentiation into myofibroblasts, initiated by transforming growth factor β and angiotensin II. This process involves the calcineurin-NFAT signaling cascade (Davis, Burr, Davis, Birnbaumer, & Molkentin, 2012), which is typically governed by frequency modulation of the ion channel-mediated Ca^{2+} signals (Kar & Parekh, 2015). Therefore, the option of introducing a defined TRPC signaling pattern using temporal precision TRPC photopharmacology might be a valuable approach to control the wound healing process and tissue repair upon injury.

Another area of active TRPC channel research is the area of cancer biology. The role of TRPC channels in tumor neovascularization, proliferation, invasion and metastasis has been investigated for a wide range of malignancies (Fels, Bulk, Pethő, & Schwab, 2018). Contribution of TRPC channels to cancer progression has been assigned to almost all members of the family including TRPC1, TRPC3, TRPC4, TRPC5 and TRPC6. The pathways underlying the cancerogenic function of TRPC channels are apparently complex. Nonetheless, Ca^{2+} entry and alteration of Ca^{2+} homeostasis are generally accepted as the pivotal initial cellular processes, and evidence for a critical role of the calcineurin/

NFAT pathway has been reported (Chigurupati et al., 2010). As alternative downstream targets, TRPC-induced Ca^{2+} influx can modulate the activity of other channels or transporters such as the Na^{+}/Ca^{2+} exchanger or chloride channels, which can subsequently impact proliferation and migration of tumor cells (Cuddapah, Turner, & Sontheimer, 2013; Jardin, Lopez, Salido, & Rosado, 2018).

In several forms of human cancer, high expression of TRPC proteins has been correlated with poor disease prognosis or poor therapeutic outcome, such as for TRPC1 in breast cancer (Azimi et al., 2017), TRPC3 in glioblastoma (Chang, Cheng, Tsai, Tsao, & Chen, 2018), TRPC5 in colon cancer (Chen et al., 2017) and TRPC6 in esophageal squamous cell carcinoma (Zhang et al., 2013) and prostate cancer (Yue, Wang, Xiao, Wang, & Ren, 2009) patients. In addition, some of the TRPC channels, including TRPC1/TRPC4/TRPC5, have recently been reported as mediators of cancer cell resistance to certain chemotherapeutics (Ma et al., 2012, 2014; Stewart, Azimi, Thompson, Roberts-Thomson, & Monteith, 2015; Wang et al., 2015; Wang et al., 2018) and to mediate adverse effects of some anticancer compounds, (Cheung et al., 2018; Muraki et al., 2017). Despite frequently reported correlations between TRPC channel expression/activity and cancer prognosis, it remains unclear and speculative whether altered TRPC expression is causally linked to disease progression. Moreover, simultaneous alterations in several TRPC channel members in the same cancer type are highly probable. For example TRPC1, TRPC3 and TRPC6 have been shown to play role in breast (Aydar, Yeo, Djamgoz, & Palmer, 2009; Diez-Bello et al., 2019; Faouzi et al., 2016; Schaar, Sukumaran, Sun, Dhasarathy, & Singh, 2016) and lung (Jiang et al., 2013; Tajeddine & Gailly, 2012; Wang et al., 2018; Yang et al., 2017) cancer as well as in glioblastoma (Chang et al., 2018; Chigurupati et al., 2010; Lepannetier et al., 2016). This indicates that successful therapy might require inhibition of multiple TRPC channel species rather than a highly selective intervention in tumors. Alternatively, altered function of TRPC channels in disease generates a specific signaling pattern that is causally linked to disease progression. Such disease-specific TRPC signaling pattern may be effectively targeted by precise photopharmacological interventions. These aspects need careful elucidation in order to clarify the value of photopharmacology.

One of the organs that display abundant TRPC expression, but at the same time represent a big challenge for the photopharmacology treatment, is the brain. Importantly, various central nervous functions have been identified to involve TRPC channels (Becker et al., 2009; Li, Xu, & Montell, 1999; Munsch, Freichel, Flockerzi, & Pape, 2003; Obukhov & Nowycky, 2002). TRPC3 has been identified as important signaling protein in cerebellar Purkinje cells and hippocampus. Mice with the gain-of-function T635A mutation of the Trpc3 gene display Purkinje cell degradation and cerebellar ataxia, known as the moonwalker phenotype (Becker et al., 2009). A more recent report suggested that the corresponding TRPC3 mutation (R762H) might also cause spinocerebellar ataxia in humans (Fogel, Hanson, & Becker, 2015). Moreover, TRPC3 channels were found to determine firing and spike frequency adaptation in murine hippocampal neurons (Neuner et al., 2015), suggesting that TRPC antagonists might be useful as neurocognitive enhancers in memory disorders. TRPC4 up-regulation as a consequence of neuronal injury has been implicated in axonal regeneration (Wu, Huang, Richardson, Priestley, & Liu, 2008). Hence, interference with neuronal TRPC signaling in a spatiotemporally precise manner is not only of value to gain understanding of the exact functions of TRPC channels in the brain, but might be indeed also considered as a therapeutic concept to specifically interfere with brain functions and to promote recovery of neuronal functions.

Evidence has also been reported for a crucial role of TRPC signaling in neurodegenerative diseases. *In vitro* studies with a neuroblastoma cells line (Bollimuntha, Singh, Shavali, Sharma, & Ebadi, 2005) and investigations in a Parkinson's disease (PD) mouse model (Selvaraj et al., 2012) demonstrated that TRPC1 might exert a protective effect in neurotoxin-induced PD, as TRPC1 knockout mice had an increased unfolded protein response and a decreased number of dopaminergic neurons. Interestingly, postmortem characterization of substantia nigra pars compacta of PD patients revealed lowered TRPC1 levels (Selvaraj et al., 2012).

Another example of neurological disorders, which are potentially related to malfunction of TRPC is epilepsy. In murine hippocampal slices, generation of group I metabotropic glutamate receptor-induced epileptiform discharges was mediated by TRPC channels (Wang, Bianchi, Chuang, Zhao, & Wong, 2007) with specific TRPC isoforms apparently exerting divergent, but synergistic roles. Whereas TRPC5 channel is responsible for the synaptic plasticity and abnormal neuron synchronization, TRPC1/TRPC4 complexes are likely involved in epileptiform burst firing by regulating firing plateau potential (Phelan et al., 2012, 2013).

In aggregate, neuronal TRPC channels have emerged as pivotal determinants of brain function and disturbances in TRPC expression and/or signaling features are likely involved in the fate and survival of neuronal cells as well as pathological dysfunction. The option of a precise and spatiotemporally well controlled manipulation of these ion channels by photopharmacology deserves consideration as a novel avenue of therapeutic intervention.

Another area where TRPC channels, especially TRPC6, have been extensively investigated are renal diseases caused by podocyte dysfunction. Importantly, TRPC6-mediated podocyte dysfunction is known to be involved in the pathophysiology of focal segmental glomerulosclerosis, as these patients have a gain-of-function mutation in the TRPC6 gene (Winn et al., 2005). Moreover, increased glucose levels have been reported to induce TRPC6 upregulation in podocytes, which suggests a role of TRPC6 in the development of diabetic nephropathy (Jardin et al., 2017; Liu et al., 2013).

An intracellular localization has been reported for almost all TRP channels (Dong, Wang, & Xu, 2010). One essential regulatory mechanism for TRP channel activity is their trafficking from intracellular compartments to the cell membrane to control transplasmalemmal Ca^{2+} transport (Toro, Arias, & Brauchi, 2011). Nonetheless, there is evidence for a role of intracellularly localized TRP channels contributing to the regulation of ion homeostasis in different organelles (Dong et al., 2010). An intracellular, perinuclear localization of TRPC has been reported by several laboratories (Borshtenbinder & Smirnov, 1975; Lavender, Chong, Ralphs, Wolstenholme, & Reaves, 2008; Treviño, Serrano, Beltrán, Felix, & Darszon, 2001). Translocation of TRPC proteins to the plasma membrane is reportedly promoted upon cell activation (Singh et al., 2004), as demonstrated for epidermal growth factor-induced translocation of TRPC3, TRPC4, TRPC5 (Bezzerides, Ramsey, Kotecha, Greka, & Clapham, 2004; Odell, Scott, & Van Helden, 2005; Smyth, Lemonnier, Vazquez, Bird, & Putney, 2006) and TRPC6 upon GPCR receptor stimulation and Ca^{2+} store depletion (Cayouette, Lussier, Mathieu, Bousquet, & Boulay, 2004). Of note, intracellular TRPC channels may represent not only a pool for the vesicular delivery of channels towards the plasma membrane. For TRPC3 and TRPC7 localization in Golgi and a role in enhanced constitutive secretion (Lavender et al., 2008) should be have been suggested. Studies in murine macrophage-like J774 cells and in alveolar macrophages form TRPC6 knockout mice suggested that TRPC6 is incorporated into the phagosomal membranes during phagocytosis to enhance phagosomal acidification (Riazanski et al., 2015). Moreover, a small fraction of TRPC3 is reportedly localized in mitochondrial membranes and interacts with mitochondrial proteins (Lockwich et al., 2008) to impact mitochondrial membrane potential and Ca^{2+} homeostasis (Feng et al., 2013). Importantly, a more recent study in a rat model of hypertension confirmed the importance of TRPC3 channel in mitochondrial function. TRPC3 expression in mitochondria purified from the vasculature of hypertensive rats was elevated, and this phenomenon was associated with increased Ca^{2+} uptake and ROS production (Wang et al., 2017).

In view of the recently accumulating evidence for critical functions of intracellular TRPC proteins, a modulation of these intracellular channel proteins by photoswitchable TRPC agonists/inhibitors needs

consideration. Control over intracellular TRPC channels requires photoswitches that are sufficiently membrane permeable in order to reach these targets. Furthermore, stability of the photoswitch in the intracellular environment is essential for effective targeting. Some azobenzene-based photoswitches have been reported as susceptible to reduction by thiols such as glutathione (Boulègue et al., 2007), which makes them barely suitable as intracellular actuators. In this case, photoswitches with incorporated S-alkyl groups in all four *ortho* positions of azobenzene, which are resistant to glutathione (Samanta et al., 2013), could be compounds of choice. There is as yet paucity of information regarding the cellular uptake of photopharmacological TRP actuators. This aspect has so far been addressed only for PhoDAGs by the Trauner laboratory. By measuring quenching of coumarin fluorescence by the azobenzene, they were able to show that different PhoDAG structures significantly differ in their ability to penetrate into the cell. The more hydrophobic PhoDAG-1 was mostly trapped in the plasma membrane, whereas PhoDAG-3 with short lipid chains was able to diffuse and accumulate within the intracellular membranes (Frank et al., 2016).

4. Conclusions

The recent advances in structural biology of TRPC channels together with rapid progress in the development of tools and methods for the control over TRPC channels by light have opened avenues to overcome the inherent hurdles in therapeutic targeting of these signaling proteins. As TRPCs serve a wide range of functions in various organ systems, cell- or tissue-specific therapeutic intervention will require either locally restricted manipulation or the development of new pharmacological tools that target a cell/phenotype-specific signaling mode of these ion channels. Technologies for light-mediated control of TRPC function based on photochromic ligands and chemogenetics are already available and expected to boost the development of innovative therapeutic concepts at the basic scientific level and by enabling efficient, all-optical screening strategies. Provided that clinically suitable methods for introduction of appropriate light or light sources into target tissues become available, it appears reasonable to anticipate successful development of TRPC photopharmacology into future clinical therapies.

Conflict of interest

All authors declare that there are no conflicts of interest.

Acknowledgement

Oleksandra Tiapko was a member of the PhD program “Metabolic and Cardiovascular Disease” which is funded by the Austrian Science Fund (FWF W1226-B18).

References

Alawi, K. M., Russell, F. A., Aubdool, A. A., Srivastava, S., Riffo-Vasquez, Y., Baldissera, L., et al. (2017). Transient receptor potential canonical 5 (TRPC5) protects against pain and vascular inflammation in arthritis and joint inflammation. *Annals of the Rheumatic Diseases 76*(1), 252–260. https://doi.org/10.1136/annrheumdis-2015-208886.

Alessandri-Haber, N., Dina, O. A., Chen, X., & Levine, J. D. (2009). TRPC1 and TRPC6 channels cooperate with TRPV4 to mediate mechanical hyperalgesia and nociceptor sensitization. *The Journal of Neuroscience 29*(19), 6217–6228. https://doi.org/10.1523/JNEUROSCI.0893-09.2009.

Alkhani, H., Ase, A. R., Grant, R., O'Donnell, D., Groschner, K., & Séguéla, P. (2014). Contribution of TRPC3 to store-operated calcium entry and inflammatory transductions in primary nociceptors. *Molecular Pain 10*, 43. https://doi.org/10.1186/1744-8069-10-43.

Aydar, E., Yeo, S., Djamgoz, M., & Palmer, C. (2009). Abnormal expression, localization and interaction of canonical transient receptor potential ion channels in human breast cancer cell lines and tissues: A potential target for breast cancer diagnosis and therapy. *Cancer Cell International 9*(1), 23. https://doi.org/10.1186/1475-2867-9-23.

Azimi, I., Milevskiy, M. J. G., Kaemmerer, E., Turner, D., Yapa, K. T. D. S., Brown, M. A., et al. (2017). TRPC1 is a differential regulator of hypoxia-mediated events and Akt signalling in PTEN-deficient breast cancer cells. *Journal of Cell Science 130*(14), 2292–2305. https://doi.org/10.1242/jcs.196659.

Azumaya, C. M., Sierra-Valdez, F., Cordero-Morales, J. F., & Nakagawa, T. (2018). Cryo-EM structure of the cytoplasmic domain of murine transient receptor potential cation channel subfamily C member 6 (TRPC6). *Journal of Biological Chemistry 293*(26), 10381–10391. https://doi.org/10.1074/jbc.RA118.003183.

Banghart, M., Borges, K., Isacoff, E., Trauner, D., & Kramer, R. H. (2004). Light-activated ion channels for remote control of neuronal firing. *Nature Neuroscience 7* (1381 EP).

Beck, B., Lehen'kyi, V., Roudbaraki, M., Flourakis, M., Charveron, M., Bordat, P., et al. (2008). TRPC channels determine human keratinocyte differentiation: New insight into basal cell carcinoma. *Cell Calcium 43*(5), 492–505. https://doi.org/10.1016/j.ceca.2007.08.005.

Becker, E. B. E., Oliver, P. L., Glitsch, M. D., Banks, G. T., Achilli, F., Hardy, A., et al. (2009). A point mutation in TRPC3 causes abnormal Purkinje cell development and cerebellar ataxia in moonwalker mice. *Proceedings of the National Academy of Sciences 106*(16), 6706–6711. https://doi.org/10.1073/pnas.0810599106.

Beech, D. J. (2013). Characteristics of transient receptor potential canonical calcium-permeable channels and their relevance to vascular physiology and disease. *Circulation Journal: Official Journal of the Japanese Circulation Society 77*(3), 570–579.

Beharry, A. A., Sadovski, O., & Woolley, G. A. (2011). Azobenzene photoswitching without ultraviolet light. *Journal of the American Chemical Society 133*(49), 19684–19687. https://doi.org/10.1021/ja209239m.

Bezzerides, V. J., Ramsey, I. S., Kotecha, S., Greka, A., & Clapham, D. E. (2004). Rapid vesicular translocation and insertion of TRP channels. *Nature Cell Biology 6*(8), 709–720. https://doi.org/10.1038/ncb1150.

Birnbaumer, L. (2015). From GTP and G proteins to TRPC channels: A personal account. *Journal of Molecular Medicine 93*(9), 941–953. https://doi.org/10.1007/s00109-015-1328-5.

Bollimuntha, S., Singh, B. B., Shavali, S., Sharma, S. K., & Ebadi, M. (2005). TRPC1-mediated inhibition of 1-methyl-4-phenylpyridinium ion neurotoxicity in human SH-SY5Y neuroblastoma cells. *Journal of Biological Chemistry 280*(3), 2132–2140. https://doi.org/10.1074/jbc.M407384200.

Borshtenbinder, I. V., & Smirnov, G. A. (1975). Results of treatment of newly detected destructive pulmonary tuberculosis. *Problemy Tuberkuleza*(11), 9–11.

Boulègue, C., Löweneck, M., Renner, C., & Moroder, L. (2007). Redox potential of azobenzene as an amino acid residue in peptides. *Chembiochem: A European Journal of Chemical Biology 8*(6), 591–594. https://doi.org/10.1002/cbic.200600495.

Brown, C., Rastogi, S. K., Barrett, S. L., Anderson, H. E., Twichell, E., Gralinski, S., et al. (2017). Differential azobenzene solubility increases equilibrium cis/trans ratio in water. *Journal of Photochemistry & Photobiology, A: Chemistry 336*, 140–145. https://doi.org/10.1016/j.jphotochem.2016.12.013.

Bush, E. W., Hood, D. B., Papst, P. J., Chapo, J. A., Minobe, W., Bristow, M. R., et al. (2006). Canonical transient receptor potential channels promote cardiomyocyte hypertrophy through activation of calcineurin signaling. *Journal of Biological Chemistry 281*(44), 33487–33496. https://doi.org/10.1074/jbc.M605536200.

Cai, S., Fatherazi, S., Presland, R. B., Belton, C. M., Roberts, F. A., Goodwin, P. C., et al. (2006). Evidence that TRPC1 contributes to calcium-induced differentiation of human keratinocytes. *Pflügers Archiv/European Journal of Physiology 452*(1), 43–52. https://doi.org/10.1007/s00424-005-0001-1.

Cao, E., Liao, M., Cheng, Y., & Julius, D. (2013). TRPV1 structures in distinct conformations reveal activation mechanisms. *Nature 504*(7478), 113–118. https://doi.org/10.1038/nature12823.

Cayouette, S., Lussier, M. P., Mathieu, E. -L., Bousquet, S. M., & Boulay, G. (2004). Exocytotic insertion of TRPC6 channel into the plasma membrane upon Gq protein-coupled receptor activation. *Journal of Biological Chemistry 279*(8), 7241–7246. https://doi.org/10.1074/jbc.M312042200.

Chang, H. -H., Cheng, Y. -C., Tsai, W. -C., Tsao, M. -J., & Chen, Y. (2018). Pyr3 induces apoptosis and inhibits migration in human glioblastoma cells. *Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology 48*(4), 1694–1702. https://doi.org/10.1159/000492293.

Chen, Z., Zhu, Y., Dong, Y., Zhang, P., Han, X., Jin, J., & Ma, X. (2017). Overexpression of TrpC5 promotes tumor metastasis via the HIF-1α-twist signaling pathway in colon cancer. *Clinical Science (London, England: 1979) 131*(19), 2439–2450. https://doi.org/10.1042/CS20171069.

Cheung, S. Y., Henrot, M., Al-Saad, M., Baumann, M., Muller, H., Unger, A., et al. (2018). TRPC4/TRPC5 channels mediate adverse reaction to the cancer cell cytotoxic agent (-)-Englerin A. *Oncotarget 9*(51), 29634–29643. https://doi.org/10.18632/oncotarget.25659.

Chigurupati, S., Venkataraman, R., Barrera, D., Naganathan, A., Madan, M., Paul, L., et al. (2010). Receptor channel TRPC6 is a key mediator of Notch-driven glioblastoma growth and invasiveness. *Cancer Research 70*(1), 418–427. https://doi.org/10.1158/0008-5472.CAN-09-2654.

Ciardo, M. G., & Ferrer-Montiel, A. (2017). Lipids as central modulators of sensory TRP channels. *Biochimica Et Biophysica Acta. Biomembranes 1859*(9 Pt B), 1615–1628. https://doi.org/10.1016/j.bbamem.2017.04.012.

Clapham, D. E. (2003). TRP channels as cellular sensors. *Nature 426*(6966), 517–524. https://doi.org/10.1038/nature02196.

Cuddapah, V. A., Turner, K. L., & Sontheimer, H. (2013). Calcium entry via TRPC1 channels activates chloride currents in human glioma cells. *Cell Calcium 53*(3), 187–194. https://doi.org/10.1016/j.ceca.2012.11.013.

Dai, Y. (2016). TRPs and pain. *Seminars in Immunopathology 38*(3), 277–291. https://doi.org/10.1007/s00281-015-0526-0.

Davis, J., Burr, A. R., Davis, G. F., Birnbaumer, L., & Molkentin, J. D. (2012). A TRPC6-dependent pathway for myofibroblast transdifferentiation and wound healing in vivo. *Developmental Cell 23*(4), 705–715. https://doi.org/10.1016/j.devcel.2012.08.017.

Dietrich, A., Mederos y Schnitzler, M., Gollasch, M., Gross, V., Storch, U., Dubrovska, G., et al. (2005). Increased vascular smooth muscle contractility in TRPC6−/− mice. *Molecular and Cellular Biology 25*(16), 6980–6989. https://doi.org/10.1128/MCB.25.16.6980-6989.2005.

Diez-Bello, R., Jardin, I., Lopez, J. J., Haouari El, M., Ortega-Vidal, J., Altarejos, J., et al. (2019). (-)-Oleocanthal inhibits proliferation and migration by modulating Ca2+ entry through TRPC6 in breast cancer cells. *Biochimica Et Biophysica Acta. Molecular Cell Research 1866*(3), 474–485. https://doi.org/10.1016/j.bbamcr.2018.10.010.

Dong, X. -P., Wang, X., & Xu, H. (2010). TRP channels of intracellular membranes. *Journal of Neurochemistry 113*(2), 313–328. https://doi.org/10.1111/j.1471-4159.2010.06626.x.

Duan, J., Li, J., Bo, Z., Chen, G. -L., Peng, X., Zhang, Y., et al. (2018). *Structure of the mouse TRPC4 ion channel. https://doi.org/10.1101/282715.*

Eichmann, T. O., & Lass, A. (2015). DAG tales: The multiple faces of diacylglycerol–stereochemistry, metabolism, and signaling. *Cellular and Molecular Life Sciences: CMLS 72*(20), 3931–3952. https://doi.org/10.1007/s00018-015-1982-3.

Estacion, M., Sinkins, W. G., Jones, S. W., Applegate, M. A. B., & Schilling, W. P. (2006). Human TRPC6 expressed in HEK 293 cells forms non-selective cation channels with limited Ca2+ permeability. *The Journal of Physiology 572*, 359–377. https://doi.org/10.1113/jphysiol.2005.103143 Pt 2.

Fan, C., Choi, W., Sun, W., Du, J., & Lu, W. (2018). Structure of the human lipid-gated cation channel TRPC3. *eLife 7*. https://doi.org/10.7554/eLife.36852 e36852–14.

Faouzi, M., Hague, F., Geerts, D., Ay, A. -S., Potier-Cartereau, M., Ahidouch, A., & Ouadid-Ahidouch, H. (2016). Functional cooperation between KCa3.1 and TRPC1 channels in human breast cancer: Role in cell proliferation and patient prognosis. *Oncotarget 7*(24), 36419–36435. https://doi.org/10.18632/oncotarget.9261.

Fehrentz, T., Schönberger, M., & Trauner, D. (2011). Optochemical genetics. *Angewandte Chemie (International Ed. in English) 50*(51), 12156–12182. https://doi.org/10.1002/anie.201103236.

Fels, B., Bulk, E., Pethő, Z., & Schwab, A. (2018). The role of TRP channels in the metastatic cascade. *Pharmaceuticals (Basel, Switzerland) 11*(2), 48. https://doi.org/10.3390/ph11020048.

Feng, S., Li, H., Tai, Y., Huang, J., Su, Y., Abramowitz, J., et al. (2013). Canonical transient receptor potential 3 channels regulate mitochondrial calcium uptake. *Proceedings of the National Academy of Sciences of the United States of America 110*(27), 11011–11016. https://doi.org/10.1073/pnas.1309531110.

Fogel, B. L., Hanson, S. M., & Becker, E. B. E. (2015). Do mutations in the murine ataxia gene TRPC3 cause cerebellar ataxia in humans? *Movement Disorders: Official Journal of the Movement Disorder Society 30*(2), 284–286. https://doi.org/10.1002/mds.26096.

Fortin, D. L., Dunn, T. W., Fedorchak, A., Allen, D., Montpetit, R., Banghart, M. R., et al. (2011). Optogenetic photochemical control of designer K+ channels in mammalian neurons. *Journal of Neurophysiology 106*(1), 488–496. https://doi.org/10.1152/jn.00251.2011.

Frank, J. A., Moroni, M., Moshourab, R., Sumser, M., Lewin, G. R., & Trauner, D. (2015). Photoswitchable fatty acids enable optical control of TRPV1. *Nature Communications 6*(1). https://doi.org/10.1038/ncomms8118 7118–11.

Frank, J. A., Yushchenko, D. A., Hodson, D. J., Lipstein, N., Nagpal, J., Rutter, G. A., et al. (2016). Photoswitchable diacylglycerols enable optical control of protein kinase C. *Nature Chemical Biology 12*(9), 755–762. https://doi.org/10.1038/nchembio.2141.

Gavva, N. R., Tamir, R., Klionsky, L., Norman, M. H., Louis, J. -C., Wild, K. D., & Treanor, J. J. S. (2005). Proton activation does not alter antagonist interaction with the capsaicin-binding pocket of TRPV1. *Molecular Pharmacology 68*(6), 1524–1533. https://doi.org/10.1124/mol.105.015727.

He, L., Zhang, Y., Ma, G., Tan, P., Li, Z., Zang, S., ... Zhou, Y. (2015a). Near-infrared photoactivatable control of Ca(2+) signaling and optogenetic immunomodulation. *eLife 4*, 1217. https://doi.org/10.7554/eLife.10024.

He, L., Zhang, Y., Ma, G., Tan, P., Li, Z., Zang, S., ... Zhou, Y. (2015b). Near-infrared photoactivatable control of Ca2+ signaling and optogenetic immunomodulation. *eLife 4*, 1217–1225. https://doi.org/10.7554/eLife.10024.

Hofmann, T., Obukhov, A. G., Schaefer, M., Harteneck, C., Gudermann, T., & Schultz, G. (1999). Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. *Nature 397*(6716), 259–263. https://doi.org/10.1038/16711.

Hofmann, T., Schaefer, M., Schultz, G., & Gudermann, T. (2002). Subunit composition of mammalian transient receptor potential channels in living cells. *Proceedings of the National Academy of Sciences 99*(11), 7461–7466. https://doi.org/10.1073/pnas.102596199.

Höglinger, D., Nadler, A., & Schultz, C. (2014). Caged lipids as tools for investigating cellular signaling. *BBA - Molecular and Cell Biology of Lipids*, 1–12. https://doi.org/10.1016/j.bbalip.2014.03.012.

Housley, G. D., Raybould, N. P., & Thorne, P. R. (1998). Fluorescence imaging of Na+ influx via P2X receptors in cochlear hair cells. *Hearing Research 119*(1–2), 1–13.

Huang, X. P., Sreekumar, R., Patel, J. R., & Walker, J. W. (1996). Response of cardiac myocytes to a ramp increase of diacylglycerol generated by photolysis of a novel caged diacylglycerol. *Biophysj 70*(5), 2448–2457. https://doi.org/10.1016/S0006-3495(96)79816-8.

Hüll, K., Morstein, J., & Trauner, D. (2018). In vivo photopharmacology. *Chemical Reviews 118*(21), 10710–10747. https://doi.org/10.1021/acs.chemrev.8b00037.

Il'ichev, Y. V., Schwörer, M. A., & Wirz, J. (2004). Photochemical reaction mechanisms of 2-Nitrobenzyl compounds: Methyl ethers and caged ATP. *Journal of the American Chemical Society 126*(14), 4581–4595. https://doi.org/10.1021/ja039071z.

Jardin, I., Lopez, J. J., Diez, R., Sánchez-Collado, J., Cantonero, C., Albarrán, L., et al. (2017). TRPs in pain sensation. *Frontiers in Physiology 8*, 392. https://doi.org/10.3389/fphys.2017.00392.

Jardin, I., Lopez, J. J., Salido, G. M., & Rosado, J. A. (2018). Store-operated Ca2+ entry in breast Cancer cells: Remodeling and functional role. *International Journal of Molecular Sciences 19*(12), 4053. https://doi.org/10.3390/ijms19124053.

Jiang, H. -N., Zeng, B., Zhang, Y., Daskoulidou, N., Fan, H., Qu, J. -M., & Xu, S. -Z. (2013). Involvement of TRPC channels in lung cancer cell differentiation and the correlation analysis in human non-small cell lung cancer. *PLoS One 8*(6), e67637. https://doi.org/10.1371/journal.pone.0067637.

Kalka, K., Merk, H., & Mukhtar, H. (2000). Photodynamic therapy in dermatology. *Journal of the American Academy of Dermatology 42*(3), 389–413– quiz 414–6.

Kamouchi, M., Philipp, S., Flockerzi, V., Wissenbach, U., Mamin, A., Raeymaekers, L., et al. (1999). Properties of heterologously expressed hTRP3 channels in bovine pulmonary artery endothelial cells. *Journal of Physiology 518*(2), 345–358.

Kar, P., & Parekh, A. B. (2015). Distinct spatial Ca2+ signatures selectively activate different NFAT transcription factor isoforms. *Molecular Cell 58*(2), 232–243. https://doi.org/10.1016/j.molcel.2015.02.027.

Kiyonaka, S., Kato, K., Nishida, M., Mio, K., Numaga, T., Sawaguchi, Y., et al. (2009). Selective and direct inhibition of TRPC3 channels underlies biological activities of a pyrazole compound. *Proceedings of the National Academy of Sciences 106*(13), 5400–5405. https://doi.org/10.1073/pnas.0808793106.

Ko, J., Myeong, J., Yang, D., & So, I. (2017). Calcium permeability of transient receptor potential canonical (TRPC) 4 channels measured by TRPC4-GCaMP6s. *The Korean Journal of Physiology & Pharmacology: Official Journal of the Korean Physiological Society and the Korean Society of Pharmacology 21*(1), 133–140. https://doi.org/10.4196/kjpp.2017.21.1.133.

Kobayashi, T., & Saito, T. (2002). Ultrafast dynamics in the excited states of Azo compounds. *Photoreactive organic thin films* (pp. 49–62). Elsevier. https://doi.org/10.1016/B978-012635490-4/50003-2.

Kokel, D., Cheung, C. Y. J., Mills, R., Coutinho-Budd, J., Huang, L., Setola, V., et al. (2013). Photochemical activation of TRPA1 channels in neurons and animals. *Nature Chemical Biology 9*(4), 257–263. https://doi.org/10.1038/nchembio.1183.

Kötter, R., Schubert, D., Dyhrfjeld-Johnsen, J., Luhmann, H. J., & Staiger, J. F. (2005). Optical release of caged glutamate for stimulation of neurons in the in vitro slice preparation. *Journal of Biomedical Optics 10*(1), 11003. https://doi.org/10.1117/1.1852555.

Lam, P. -Y., Mendu, S. K., Mills, R. W., Zheng, B., Padilla, H., Milan, D. J., et al. (2017). A high-conductance chemo-optogenetic system based on the vertebrate channel Trpa1b. *Scientific Reports 7*(1), 11839. https://doi.org/10.1038/s41598-017-11791-z.

Lavender, V., Chong, S., Ralphs, K., Wolstenholme, A. J., & Reaves, B. J. (2008). Increasing the expression of calcium-permeable TRPC3 and TRPC7 channels enhances constitutive secretion. *The Biochemical Journal 413*(3), 437–446. https://doi.org/10.1042/BJ20071488.

Lee, C. -H., Wu, S. -B., Hong, C. -H., Yu, H. -S., & Wei, Y. -H. (2013). Molecular mechanisms of UV-induced apoptosis and its effects on skin residential cells: The implication in UV-based phototherapy. *International Journal of Molecular Sciences 14*(3), 6414–6435. https://doi.org/10.3390/ijms14036414.

Leinders-Zufall, T., Storch, U., Bleymehl, K., Mederos y schnitzler, M., Frank, J. A., Konrad, D. B., et al. (2018). PhoDAGs enable optical control of diacylglycerol-sensitive transient receptor potential channels. *Cell Chemical Biology 25*(2), 215–223 e3 https://doi.org/10.1016/j.chembiol.2017.11.008.

Lepannetier, S., Zanou, N., Yerna, X., Emeriau, N., Dufour, I., Masquelier, J., et al. (2016). Sphingosine-1-phosphate-activated TRPC1 channel controls chemotaxis of glioblastoma cells. *Cell Calcium 60*(6), 373–383. https://doi.org/10.1016/j.ceca.2016.09.002.

Lerch, M. M., Hansen, M. J., van Dam, G. M., Szymanski, W., & Feringa, B. L. (2016). Emerging targets in photopharmacology. *Angewandte Chemie (International Ed. in English) 55*(37), 10978–10999. https://doi.org/10.1002/anie.201601931.

Leuner, K., Kraus, M., Woelfle, U., Beschmann, H., Harteneck, C., Boehncke, W. -H., et al. (2011). Reduced TRPC channel expression in psoriatic keratinocytes is associated with impaired differentiation and enhanced proliferation. *PLoS One 6*(2), e14716. https://doi.org/10.1371/journal.pone.0014716.

Li, H. S., Xu, X. Z., & Montell, C. (1999). Activation of a TRPC3-dependent cation current through the neurotrophin BDNF. *Neuron 24*(1), 261–273.

Lichtenegger, M., & Groschner, K. (2014). TRPC3: A multifunctional signaling molecule. *Handbook of Experimental Pharmacology 222*(Chapter 4), 67–84. https://doi.org/10.1007/978-3-642-54215-2_4.

Lichtenegger, M., Tiapko, O., Svobodova, B., Stockner, T., Glasnov, T. N., Schreibmayer, W., et al. (2018). An optically controlled probe identifies lipid-gating fenestrations within the TRPC3 channel. *Nature Chemical Biology 14*(4), 1–9. https://doi.org/10.1038/s41589-018-0015-6.

Liu, B. -C., Song, X., Lu, X. -Y., Li, D. T., Eaton, D. C., Shen, B. -Z., et al. (2013). High glucose induces podocyte apoptosis by stimulating TRPC6 via elevation of reactive oxygen species. *Biochimica et Biophysica Acta 1833*(6), 1434–1442. https://doi.org/10.1016/j.bbamcr.2013.02.031.

Lockwich, T., Pant, J., Makusky, A., Jankowska-Stephens, E., Kowalak, J. A., Markey, S. P., & Ambudkar, I. S. (2008). Analysis of TRPC3-interacting proteins by tandem mass spectrometry. *Journal of Proteome Research 7*(3), 979–989. https://doi.org/10.1021/pr070496k.

Lutas, A., Birnbaumer, L., & Yellen, G. (2014). Metabolism regulates the spontaneous firing of substantia nigra pars reticulata neurons via KATP and nonselective cation channels. *The Journal of Neuroscience 34*(49), 16336–16347. https://doi.org/10.1523/JNEUROSCI.1357-14.2014.

Ma, X., Cai, Y., He, D., Zou, C., Zhang, P., Lo, C. Y., et al. (2012). Transient receptor potential channel TRPC5 is essential for P-glycoprotein induction in drug-resistant cancer cells. *Proceedings of the National Academy of Sciences of the United States of America 109*(40), 16282–16287. https://doi.org/10.1073/pnas.1202989109.

Ma, X., Chen, Z., Hua, D., He, D., Wang, L., Zhang, P., et al. (2014). Essential role for TrpC5-containing extracellular vesicles in breast cancer with chemotherapeutic resistance. *Proceedings of the National Academy of Sciences of the United States of America 111*(17), 6389–6394. https://doi.org/10.1073/pnas.1400272111.

McGirt, L. Y., Vasagar, K., Gober, L. M., Saini, S. S., & Beck, L. A. (2006). Successful treatment of recalcitrant chronic idiopathic urticaria with sulfasalazine. *Archives of Dermatology 142*(10), 1337–1342. https://doi.org/10.1001/archderm.142.10.1337.

Miller, M., Shi, J., Zhu, Y., Kustov, M., Tian, J. -B., Stevens, A., et al. (2011). Identification of ML204, a novel potent antagonist that selectively modulates native TRPC4/C5 ion channels. *Journal of Biological Chemistry 286*(38), 33436–33446. https://doi.org/10.1074/jbc.M111.274167.

Minke, B., & Cook, B. (2002). TRP channel proteins and signal transduction. *Physiological Reviews 82*(2), 429–472. https://doi.org/10.1152/physrev.00001.2002.

Montgomery, K. L., Yeh, A. J., Ho, J. S., Tsao, V., Mohan Iyer, S., Grosenick, L., et al. (2015). Wirelessly powered, fully internal optogenetics for brain, spinal and peripheral circuits in mice. *Nature Methods 12*(10), 969–974. https://doi.org/10.1038/nmeth.3536.

Motoyama, K., Nagata, T., Kobayashi, J., Nakamura, A., Miyoshi, N., Kazui, M., et al. (2018). Discovery of a bicyclo[4.3.0]nonane derivative DS88790512 as a potent, selective, and orally bioavailable blocker of transient receptor potential canonical 6 (TRPC6). *Bioorganic & Medicinal Chemistry Letters 28*(12), 2222–2227. https://doi.org/10.1016/j.bmcl.2018.03.056.

Müller, M., Essin, K., Hill, K., Beschmann, H., Rubant, S., Schempp, C. M., et al. (2008). Specific TRPC6 channel activation, a novel approach to stimulate keratinocyte differentiation. *Journal of Biological Chemistry 283*(49), 33942–33954. https://doi.org/10.1074/jbc.M801844200.

Munsch, T., Freichel, M., Flockerzi, V., & Pape, H. -C. (2003). Contribution of transient receptor potential channels to the control of GABA release from dendrites. *Proceedings of the National Academy of Sciences 100*(26), 16065–16070. https://doi.org/10.1073/pnas.2535311100.

Muraki, K., Ohnishi, K., Takezawa, A., Suzuki, H., Hatano, N., Muraki, Y., et al. (2017). Na+ entry through heteromeric TRPC4/C1 channels mediates (-)Englerin A-induced cytotoxicity in synovial sarcoma cells. *Scientific Reports 7*(1), 16988. https://doi.org/10.1038/s41598-017-17303-3.

Nadler, A., Reither, G., Feng, S., Stein, F., Reither, S., Müller, R., & Schultz, C. (2013). The fatty acid composition of diacylglycerols determines local signaling patterns. *Angewandte Chemie (International Ed. in English) 52*(24), 6330–6334. https://doi.org/10.1002/anie.201301716.

Neuner, S. M., Wilmott, L. A., Hope, K. A., Hoffmann, B., Chong, J. A., Abramowitz, J., et al. (2015). TRPC3 channels critically regulate hippocampal excitability and contextual fear memory. *Behavioural Brain Research 281*, 69–77. https://doi.org/10.1016/j.bbr.2014.12.018.

Nilius, B., & Flockerzi, V. (2014). Mammalian transient receptor potential (TRP) cation channels. *Preface. Handbook of experimental pharmacology. 223*. (v–vi).

Obukhov, A. G., & Nowycky, M. C. (2002). TRPC4 can be activated by G-protein-coupled receptors and provides sufficient Ca(2+) to trigger exocytosis in neuroendocrine cells. *Journal of Biological Chemistry 277*(18), 16172–16178. https://doi.org/10.1074/jbc.M111664200.

Odell, A. F., Scott, J. L., & Van Helden, D. F. (2005). Epidermal growth factor induces tyrosine phosphorylation, membrane insertion, and activation of transient receptor potential channel 4. *Journal of Biological Chemistry 280*(45), 37974–37987. https://doi.org/10.1074/jbc.M503646200.

Park, S. I., Brenner, D. S., Shin, G., Morgan, C. D., Copits, B. A., Chung, H. U., et al. (2015). Soft, stretchable, fully implantable miniaturized optoelectronic systems for wireless optogenetics. *Nature Biotechnology 33*(12), 1280–1286. https://doi.org/10.1038/nbt.3415.

Phelan, K. D., Mock, M. M., Kretz, O., Shwe, U. T., Kozhemyakin, M., Greenfield, L. J., et al. (2012). Heteromeric canonical transient receptor potential 1 and 4 channels play a critical role in epileptiform burst firing and seizure-induced neurodegeneration. *Molecular Pharmacology 81*(3), 384–392. https://doi.org/10.1124/mol.111.075341.

Phelan, K. D., Shwe, U. T., Abramowitz, J., Wu, H., Rhee, S. W., Howell, M. D., et al. (2013). Canonical transient receptor channel 5 (TRPC5) and TRPC1/4 contribute to seizure and excitotoxicity by distinct cellular mechanisms. *Molecular Pharmacology 83*(2), 429–438. https://doi.org/10.1124/mol.112.082271.

Poteser, M., Graziani, A., Eder, P., Yates, A., Mächler, H., Romanin, C., & Groschner, K. (2008). Identification of a rare subset of adipose tissue-resident progenitor cells, which express CD133 and TRPC3 as a VEGF-regulated Ca 2+entry channel. *FEBS Letters 582*(18), 2696–2702. https://doi.org/10.1016/j.febslet.2008.06.049.

Poteser, M., Graziani, A., Rosker, C., Eder, P., Derler, I., Kahr, H., et al. (2006). TRPC3 and TRPC4 associate to form a redox-sensitive cation channel. Evidence for expression of native TRPC3-TRPC4 heteromeric channels in endothelial cells. *Journal of Biological Chemistry 281*(19), 13588–13595. https://doi.org/10.1074/jbc.M512205200.

Poteser, M., Schleifer, H., Lichtenegger, M., Schernthaner, M., Stockner, T., Kappe, C. O., et al. (2011). PKC-dependent coupling of calcium permeation through transient receptor potential canonical 3 (TRPC3) to calcineurin signaling in HL-1 myocytes. *Proceedings of the National Academy of Sciences of the United States of America 108*(26), 10556–10561. https://doi.org/10.1073/pnas.1106183108.

Qu, L., Li, Y., Pan, X., Zhang, P., LaMotte, R. H., & Ma, C. (2012). Transient receptor potential canonical 3 (TRPC3) is required for IgG immune complex-induced excitation of the rat dorsal root ganglion neurons. *The Journal of Neuroscience 32*(28), 9554–9562. https://doi.org/10.1523/JNEUROSCI.6355-11.2012.

Quann, E. J., Merino, E., Furuta, T., & Huse, M. (2009). Localized diacylglycerol drives the polarization of the microtubule-organizing center in T cells. *Nature Immunology 10*(6), 627–635. https://doi.org/10.1038/ni.1734.

Rial Verde, E. M., Zayat, L., Etchenique, R., & Yuste, R. (2008). Photorelease of GABA with visible light using an inorganic caging group. *Frontiers in Neural Circuits 2*, 2. https://doi.org/10.3389/neuro.04.002.2008.

Riazanski, V., Gabdoulkhakova, A. G., Boynton, L. S., Eguchi, R. R., Deriy, L. V., Hogarth, D. K., et al. (2015). TRPC6 channel translocation into phagosomal membrane augments phagosomal function. *Proceedings of the National Academy of Sciences of the United States of America 112*(47), E6486–E6495. https://doi.org/10.1073/pnas.1518966112.

Rubaiy, H. N., Ludlow, M. J., Bon, R. S., & Beech, D. J. (2017). Pico145 - powerful new tool for TRPC1/4/5 channels. *Channels (Austin, Tex.) 11*(5), 362–364. https://doi.org/10.1080/19336950.2017.1317485.

Rubaiy, H. N., Ludlow, M. J., Henrot, M., Gaunt, H. J., Miteva, K., Cheung, S. Y., et al. (2017). Picomolar, selective, and subtype-specific small-molecule inhibition of TRPC1/4/5 channels. *Journal of Biological Chemistry 292*(20), 8158–8173. https://doi.org/10.1074/jbc.M116.773556.

Samanta, S., McCormick, T. M., Schmidt, S. K., Seferos, D. S., & Woolley, G. A. (2013). Robust visible light photoswitching with ortho-thiol substituted azobenzenes. *Chemical Communications (Cambridge, England) 49*(87), 10314–10316. https://doi.org/10.1039/c3cc46045b.

Samanta, S., Qin, C., Lough, A. J., & Woolley, G. A. (2012). Bidirectional photocontrol of peptide conformation with a bridged azobenzene derivative. *Angewandte Chemie (International Ed. in English) 51*(26), 6452–6455. https://doi.org/10.1002/anie.201202383.

Schaar, A., Sukumaran, P., Sun, Y., Dhasarathy, A., & Singh, B. B. (2016). TRPC1-STIM1 activation modulates transforming growth factor β-induced epithelial-to-mesenchymal transition. *Oncotarget 7*(49), 80554–80567. https://doi.org/10.18632/oncotarget.12895.

Schleifer, H., Doleschal, B., Lichtenegger, M., Oppenrieder, R., Derler, I., Frischauf, I., et al. (2012). Novel pyrazole compounds for pharmacological discrimination between receptor-operated and store-operated Ca 2+entry pathways. *British Journal of Pharmacology 167*(8), 1712–1722. https://doi.org/10.1111/j.1476-5381.2012.02126.x.

Selvaraj, S., Sun, Y., Watt, J. A., Wang, S., Lei, S., Birnbaumer, L., & Singh, B. B. (2012). Neurotoxin-induced ER stress in mouse dopaminergic neurons involves downregulation of TRPC1 and inhibition of AKT/mTOR signaling. *The Journal of Clinical Investigation 122*(4), 1354–1367. https://doi.org/10.1172/JCI61332.

Seo, K., Rainer, P. P., Shalkey Hahn, V., Lee, D. I., Jo, S. H., Andersen, A., et al. (2014). Combined TRPC3 and TRPC6 blockade by selective small-molecule or genetic deletion inhibits pathological cardiac hypertrophy. *Proceedings of the National Academy of Sciences 111*(4), 1551–1556. https://doi.org/10.1073/pnas.1308963111.

Sierra-Valdez, F., Azumaya, C. M., Romero, L. O., Nakagawa, T., & Cordero-Morales, J. F. (2018). Structure-function analyses of the ion channel TRPC3 reveal that its cytoplasmic domain allosterically modulates channel gating. *Journal of Biological Chemistry 293*(41), 16102–16114. https://doi.org/10.1074/jbc.RA118.005066.

Siewertsen, R., Neumann, H., Buchheim-Stehn, B., Herges, R., Näther, C., Renth, F., & Temps, F. (2009). Highly efficient reversible Z-E photoisomerization of a bridged azobenzene with visible light through resolved S(1)(n pi*) absorption bands. *Journal of the American Chemical Society 131*(43), 15594–15595. https://doi.org/10.1021/ja906547d.

Singh, B. B., Lockwich, T. P., Bandyopadhyay, B. C., Liu, X., Bollimuntha, S., Brazer, S. -C., et al. (2004). VAMP2-dependent exocytosis regulates plasma membrane insertion of TRPC3 channels and contributes to agonist-stimulated Ca2+ influx. *Molecular Cell 15*(4), 635–646. https://doi.org/10.1016/j.molcel.2004.07.010.

Smyth, J. T., Lemonnier, L., Vazquez, G., Bird, G. S., & Putney, J. W. (2006). Dissociation of regulated trafficking of TRPC3 channels to the plasma membrane from their activation by phospholipase C. *Journal of Biological Chemistry 281*(17), 11712–11720. https://doi.org/10.1074/jbc.M510541200.

Stein, M., Breit, A., Fehrentz, T., Gudermann, T., & Trauner, D. (2013). Optical control of TRPV1 channels. *Angewandte Chemie (International Ed. in English) 52*(37), 9845–9848. https://doi.org/10.1002/anie.201302530.

Sternson, S. M., & Roth, B. L. (2014). Chemogenetic tools to interrogate brain functions. *Annual Review of Neuroscience 37*(1), 387–407. https://doi.org/10.1146/annurev-neuro-071013-014048.

Stewart, T. A., Azimi, I., Thompson, E. W., Roberts-Thomson, S. J., & Monteith, G. R. (2015). A role for calcium in the regulation of ATP-binding cassette, sub-family C, member 3 (ABCC3) gene expression in a model of epidermal growth factor-mediated breast cancer epithelial-mesenchymal transition. *Biochemical and Biophysical Research Communications 458*(3), 509–514. https://doi.org/10.1016/j.bbrc.2015.01.141.

Storch, U., Forst, A. -L., Pardatscher, F., Erdogmus, S., Philipp, M., Gregoritza, M., et al. (2017). Dynamic NHERF interaction with TRPC4/5 proteins is required for channel gating by diacylglycerol. *Proceedings of the National Academy of Sciences of the United States of America 114*(1), E37–E46. https://doi.org/10.1073/pnas.1612263114.

Storch, U., Forst, A. -L., Philipp, M., Gudermann, T., & Mederos y Schnitzler, M. (2012). Transient receptor potential channel 1 (TRPC1) reduces calcium permeability in heteromeric channel complexes. *Journal of Biological Chemistry 287*(5), 3530–3540. https://doi.org/10.1074/jbc.M111.283218.

Sunshine, H., & Iruela-Arispe, M. L. (2017). Membrane lipids and cell signaling. *Current Opinion in Lipidology 28*(5), 408–413. https://doi.org/10.1097/MOL.0000000000000443.

Svobodova, B., Lichtenegger, M., Platzer, D., Di Giuro, C. M. L., Cruz, l., de, G. G., Glasnov, T., et al. (2019). A single point mutation in the TRPC3 lipid-recognition window generates supersensitivity to benzimidazole channel activators. *Cell Calcium 79*, 27–34. https://doi.org/10.1016/j.ceca.2019.02.007.

Tai, Y., Yang, S., Liu, Y., & Shao, W. (2017). TRPC channels in health and disease. *Advances in Experimental Medicine and Biology 976*(11), 35–45. https://doi.org/10.1007/978-94-024-1088-4_4.

Tajeddine, N., & Gailly, P. (2012). TRPC1 protein channel is major regulator of epidermal growth factor receptor signaling. *Journal of Biological Chemistry 287*(20), 16146–16157. https://doi.org/10.1074/jbc.M112.340034.

Tang, Q., Guo, W., Zheng, L., Wu, J. -X., Liu, M., Zhou, X., et al. (2018). Structure of the receptor-activated human TRPC6 and TRPC3 ion channels. *Cell Research 28*(7), 1–10. https://doi.org/10.1038/s41422-018-0038-2.

Than, J. Y. -X. L., Li, L., Hasan, R., & Zhang, X. (2013). Excitation and modulation of TRPA1, TRPV1, and TRPM8 channel-expressing sensory neurons by the pruritogen chloroquine. *Journal of Biological Chemistry 288*(18), 12818–12827. https://doi.org/10.1074/jbc.M113.450072.

Tiapko, O., Bacsa, B., Cruz, l., de, G. G., Glasnov, T., & Groschner, K. (2016). Optopharmacological control of TRPC channels by coumarin-caged lipids is associated with a phototoxic membrane effect. *Science China. Life Sciences 59*(8), 802–810. https://doi.org/10.1007/s11427-016-5095-5.

Tiapko, O., & Groschner, K. (2018). TRPC3 as a target of novel therapeutic interventions. *Cells 7*(7), 83. https://doi.org/10.3390/cells7070083.

Tiapko, O., Shrestha, N., Lindinger, S., Guedes de la Cruz, G., Graziani, A., Klec, C., et al. (2019). Lipid-independent control of endothelial and neuronal TRPC3 channels by light. *Chemical Science*. https://doi.org/10.1039/C8SC05536J.

Toro, C. A., Arias, L. A., & Brauchi, S. (2011). Sub-cellular distribution and translocation of TRP channels. *Current Pharmaceutical Biotechnology 12*(1), 12–23.

Treviño, C. L., Serrano, C. J., Beltrán, C., Felix, R., & Darszon, A. (2001). Identification of mouse trp homologs and lipid rafts from spermatogenic cells and sperm. *FEBS Letters 509*(1), 119–125.

Velema, W. A., Szymanski, W., & Feringa, B. L. (2014). Photopharmacology: Beyond proof of principle. *Journal of the American Chemical Society 136*(6), 2178–2191. https://doi.org/10.1021/ja413063e.

Vinayagam, D., Mager, T., Apelbaum, A., Bothe, A., Merino, F., Hofnagel, O., et al. (2018). Electron cryo-microscopy structure of the canonical TRPC4 ion channel. *eLife 7*, 213. https://doi.org/10.7554/eLife.36615.

Wajdner, H. E., Farrington, J., Barnard, C., Peachell, P. T., Schnackenberg, C. G., Marino, J. P., et al. (2017). Orai and TRPC channel characterization in FcεRI-mediated calcium signaling and mediator secretion in human mast cells. *Physiological Reports 5*(5), e13166. https://doi.org/10.14814/phy2.13166.

Wang, B., Xiong, S., Lin, S., Xia, W., Li, Q., Zhao, Z., et al. (2017). Enhanced mitochondrial transient receptor potential channel, canonical type 3-mediated calcium handling in the vasculature from hypertensive rats. *Journal of the American Heart Association 6*(7). https://doi.org/10.1161/JAHA.117.005812.

Wang, F., Smith, N. A., Xu, Q., Goldman, S., Peng, W., Huang, J. H., et al. (2013). Photolysis of caged Ca2+ but not receptor-mediated Ca2+ signaling triggers astrocytic glutamate release. *The Journal of Neuroscience 33*(44), 17404–17412. https://doi.org/10.1523/JNEUROSCI.2178-13.2013.

Wang, M., Bianchi, R., Chuang, S. -C., Zhao, W., & Wong, R. K. S. (2007). Group I metabotropic glutamate receptor-dependent TRPC channel trafficking in hippocampal neurons. *Journal of Neurochemistry 101*(2), 411–421. https://doi.org/10.1111/j.1471-4159.2006.04377.x.

Wang, T., Chen, Z., Zhu, Y., Pan, Q., Liu, Y., Qi, X., et al. (2015). Inhibition of transient receptor potential channel 5 reverses 5-fluorouracil resistance in human colorectal cancer cells. *Journal of Biological Chemistry 290*(1), 448–456. https://doi.org/10.1074/jbc.M114.590364.

Wang, T., Ning, K., Sun, X., Zhang, C., Jin, L. -F., & Hua, D. (2018). Glycolysis is essential for chemoresistance induced by transient receptor potential channel C5 in colorectal cancer. *BMC Cancer 18*(1), 207. https://doi.org/10.1186/s12885-018-4123-1.

Wang, Y., He, J., Jiang, H., Zhang, Q., Yang, H., Xu, X., et al. (2018). Nicotine enhances store-operated calcium entry by upregulating HIF-1α and SOCC components in non-small cell lung cancer cells. *Oncology Reports 40*(4), 2097–2104. https://doi.org/10.3892/or.2018.6580.

Washburn, D. G., Holt, D. A., Dodson, J., McAtee, J. J., Terrell, L. R., Barton, L., et al. (2013). The discovery of potent blockers of the canonical transient receptor channels, TRPC3 and TRPC6, based on an anilino-thiazole pharmacophore. *Bioorganic & Medicinal Chemistry Letters 23*(17), 4979–4984. https://doi.org/10.1016/j.bmcl.2013.06.047.

Weissleder, R., & Ntziachristos, V. (2003). Shedding light onto live molecular targets. *Nature Medicine 9*(1), 123–128. https://doi.org/10.1038/nm0103-123.

Winn, M. P., Conlon, P. J., Lynn, K. L., Farrington, M. K., Creazzo, T., Hawkins, A. F., et al. (2005). A mutation in the TRPC6 cation channel causes familial focal segmental glomerulosclerosis. *Science 308*(5729), 1801–1804. https://doi.org/10.1126/science.1106215.

Wu, D., Huang, W., Richardson, P. M., Priestley, J. V., & Liu, M. (2008). TRPC4 in rat dorsal root ganglion neurons is increased after nerve injury and is necessary for neurite outgrowth. *Journal of Biological Chemistry 283*(1), 416–426. https://doi.org/10.1074/jbc.M703177200.

Xu, X., Lozinskaya, I., Costell, M., Lin, Z., Ball, J. A., Bernard, R., et al. (2013). *Biophysj 104*(S1), 454a. https://doi.org/10.1016/j.bpj.2012.11.2513 Characterization of small molecule TRPC3 and TRPC6 agonist and antagonists.

Yang, Z., He, J., Gao, P., Niu, Y., Zhang, J., Wang, L., et al. (2017). miR-769-5p suppressed cell proliferation, migration and invasion by targeting TGFBR1 in non-small cell lung carcinoma. *Oncotarget 8*(69), 113558–113570. https://doi.org/10.18632/oncotarget.23060.

Yue, D., Wang, Y., Xiao, J. -Y., Wang, P., & Ren, C. -S. (2009). Expression of TRPC6 in benign and malignant human prostate tissues. *Asian Journal of Andrology 11*(5), 541–547. https://doi.org/10.1038/aja.2009.53.

Zbaida, S. (1995). The mechanism of microsomal azoreduction: Predictions based on electronic aspects of structure-activity relationships. *Drug Metabolism Reviews 27*(3), 497–516. https://doi.org/10.3109/03602539508998333.

Zhang, S. -S., Wen, J., Yang, F., Cai, X. -L., Yang, H., Luo, K. -J., et al. (2013). High expression of transient potential receptor C6 correlated with poor prognosis in patients with esophageal squamous cell carcinoma. *Medical Oncology (Northwood, London, England) 30*(3), 607. https://doi.org/10.1007/s12032-013-0607-7.

Zhu, X., Jiang, M., & Birnbaumer, L. (1998). Receptor-activated Ca2+ influx via human Trp3 stably expressed in human embryonic kidney (HEK)293 cells. Evidence for a non-capacitative Ca2+ entry. *Journal of Biological Chemistry 273*(1), 133–142.

Zhu, X., Jiang, M., Peyton, M., Boulay, G., Hurst, R., Stefani, E., & Birnbaumer, L. (1996). trp, A novel mammalian gene family essential for agonist-activated capacitative Ca2+ entry. *Cell 85*(5), 661–671.