A Transient Receptor Potential Ion Channel in *Chlamydomonas* Shares Key Features with Sensory Transduction-Associated TRP Channels in Mammals

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Sensory modalities are essential for navigating through an ever-changing environment. From insects to mammals, transient receptor potential (TRP) channels are known mediators for cellular sensing. *Chlamydomonas reinhardtii* is a motile single-celled freshwater green alga that is guided by photosensory, mechanosensory, and chemosensory cues. In this type of alga, sensory input is first detected by membrane receptors located in the cell body and then transduced to the beating cilia by membrane depolarization. Although TRP channels seem to be absent in plants, *C. reinhardtii* possesses genomic sequences encoding TRP proteins. Here, we describe the cloning and characterization of a *C. reinhardtii* version of a TRP channel sharing key features present in mammalian TRP channels associated with sensory transduction. In silico sequence-structure analysis unveiled the modular design of TRP channels, and electrophysiological experiments conducted on Human Embryonic Kidney-293T cells expressing the Cr-TRP1 clone showed that many of the core functional features of metazoan TRP channels are present in Cr-TRP1, suggesting that basic TRP channel gating characteristics evolved early in the history of eukaryotes.

INTRODUCTION

The transient receptor potential (TRP) channel family of cation channels is diverse in terms of structure, ion selectivity, activation mechanisms, and tissue distribution (Clapham, 2009). In mammals, the TRP family comprises 28 loosely related ion channel proteins that are classified into six subfamilies (Latorre et al., 2009). Mammalian TRP channel proteins are polyomodal cation channels that participate in sensory physiology at different levels. These include thermosensation, mechanosensation, nociception (sensation of noxious stimuli), touch, taste, olfaction, and vision (Clapham, 2008; Latorre et al., 2009). Under physiological conditions, TRP channel opening allows for the fast entrance of sodium and calcium ions into the cell (Owsianik et al., 2006). Although originally found in *Drosophila melanogaster* (Cosens and Manning, 1969; Montell and Rubin, 1989), at present, TRP channels are mostly studied in mammalian cells. TRPY, from yeast vacuole, is the only TRP channel from a unicellular organism that has been cloned and described to date (Martincic et al., 2008). After the release of the *Chlamydomonas reinhardtii* genome sequence, more than 60 putative ion channels, including TRP channels, have been reported as probable gene products (Merchant et al., 2007). Commonly found in soil and freshwater, *C. reinhardtii* is a single-celled chlorophyte alga about 10 μm in diameter with two beating flagella that enable swimming with a breast-stroke-type motion (Harris, 2001). Navigating at ~50 μm/s (Harris, 2001), these algae must rapidly integrate multiple external cues to adjust their orientation relative to the source of the signal. Notably, *C. reinhardtii* possesses a finely tuned navigation system based on calcium conductances (Hegemann, 2008). The presence of putative TRP channel coding sequences in the *C. reinhardtii* genome makes them good candidates for both the generation of the input signal and/or the regulation of sensory input propagation. Two recent independent studies report behavioral changes after knocking down TRP channel transcripts in *Chlamydomonas*. Apparently expressed at the flagella, silencing the expression of TRPP2 reduces the phosphorylation of cyclic GMP-dependent protein kinase and affects algae mating behavior (Huang et al., 2007). On the other hand, TRPV-related TRP11 (also expressed at the flagella) has been associated with the mechanosensory response (Fujii et al., 2011). Unfortunately, in both cases, neither channel heterologous expression nor algal electrical activity was described, hampering the correct interpretation of the behavioral data. Here, we present a novel functional TRP channel from *C. reinhardtii* with a predicted molecular architecture that combines features from different TRP channel subfamilies. Of equal importance, this TRP channel displays several functional properties present in TRP channels from multicellular organisms, such as outward rectification, weak voltage dependence, phosphatidylinositol 4,5-bisphosphate (PIP2) sensitization, pharmacological block by N-(4-fluoro-3-butoxy)phenyl)-4-(3-[[1-(2H)-carboxamide (BCTC), and activation by temperature (Latorre et al., 2009).
RESULSTES

Identification of TRP-Like Channels from C. reinhardtii

A previous report identified eight putative TRP channels in C. reinhardtii (Fujiu et al., 2011). Our bioinformatics analysis recognized six of these channels as aligning well with the full lengths as well as the transmembrane regions of bona fide TRP channels: Chlamydomonas TRP1, TRP2, TRP11, TRP13, TRP16, and TRPP2. Of the other two channels identified previously, we found that TRP5 was homologous to some TRP channels but did not align well with the transmembrane region of C. reinhardtii (Fujiu et al., 2011). Our bioinformatics analysis identified four additional TRP channels in C. reinhardtii: one already identified in Phytozome as a TRP channel, TRP6, and three others not annotated in Phytozome, named TRP21, TRP22, and TRP23. From the whole subset, and based on its particular predicted architecture, we decided to focus our attention on TRP1.

Localization of Cr-TRP1 within the TRP Family and Inferred Phylogeny

In order to classify Cr-TRP1 within the context of the entire TRP group of proteins, we created a sequence similarity network (SSN; Atkinson et al., 2009). SSNs are based on all-against-all sequence comparisons and, like other networks, are very robust to missing data. Furthermore, they allow easy identification of clades and correlate well with phylogenetic trees (Atkinson et al., 2009; Brown and Babbitt, 2012; Lukk et al., 2012). The network was first built considering the 67 TRP channels annotated in the International Union of Basic and Clinical Pharmacology database encompassing TRP subfamilies A (ankyrin), C (classical or canonical), M (melastatin), ML (mucolipin), P (polycystin), and V (vanilloid) (http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=78), with the addition of No mechanoreceptor potential C (NompC) from Drosophila belonging to the TRPN (NompC-like) subfamily (Cheng et al., 2010), as seed sequences. Then, homologs from the National Center for Biotechnology Information (NCBI) database having a significant BLASTP E-value hit against at least two of the seed sequences, and which align with at least 70% of the transmembrane region of one of the seeds, were incorporated into the network. We used the same procedure to identify putative TRP channels in 23 different algae and unicellular organisms (see Methods). In total, we identified 7126 proteins that show high similarity to known TRP family members. Figure 1A shows an SSN of 2841 representatives assembled at the permissive E-value threshold of 1e^-18 (median alignment length of 651 residues, median identity of 35.8%). Here, all seven functional TRP families are already clearly defined (six mammalian subfamilies plus TRPN). At this level, TRPPs and TRPMLs are not connected to the rest of the subfamilies or to each other. In this network, Cr-TRP1 remains attached to a cluster including members in the TRPA, TRPC, TRPM, TRPN, and TRPV families. At a more stringent E-value threshold of 1e^-87, an SSN of all 7126 proteins of the TRP group (Figure 1B; median alignment length of 812 residues, median identity of 48%) shows all seven currently characterized functional subfamilies separated from each other, as expected. In this network, Cr-TRP1 is isolated from known families and only connected to a TRP channel from Volvox carteri (a close multicellular relative of C. reinhardtii; Prochnik et al., 2010). Thus, according to the SSN using several known extant TRP channels and their close homologs, it appears that Cr-TRP1 is a true member of the TRP ion channel superfamily (Figure 1A). Yet, it does not show enough similarity to members of the seven characterized subfamilies and is instead isolated inside its own network (Figure 1B). Consequently, it seems that Cr-TRP1 belongs to a novel functional TRP family.

To investigate the evolutionary relationships between Cr-TRP1 and the rest of the members of the TRP family, a phylogenetic reconstruction was performed using a Bayesian method and the Jones substitution model (Jones et al., 1992). The phylogenetic tree includes the 68 functionally characterized channels from the SSNs plus all 33 putative TRP channels identified in the complete genomes of algae and unicellular organisms (Figures 2; Supplemental Data Set 1). Figure 2 shows a clustering pattern similar to those seen in the SSNs (i.e., the TRP family contains two evolutionarily distinct clusters: one composed of TRPAs, TRPCs, TRPMs, TRPNs and TRPVs, to which Cr-TRP1 belongs [cluster A/C/M/N/V]), and the other composed of TRPPs and TRPMLs [cluster P/ML]). Interestingly, most of the 33 putative TRP channels from algae and unicellular organisms seem to have diverged from a common ancestor together with the TRPP and TRPML channels. The P/ML cluster includes Cr-TRPP2, as expected, but also Cr-TRP11, which was reported previously to be a distant relative of the TRPV family (Fujiu et al., 2011). The phylogenetic tree shows that Cr-TRP1 is closely related to a TRP channel in V. carteri as well as to two other paralogous channels in C. reinhardtii. These four channels seem to have diverged from a common ancestor, along with the TRPC, TRPM, and TRPN channels, at a time when the TRPA and TRPV channels had already diverged. These four channels represent the only TRP proteins from algae or unicellular organisms in the A/C/M/N/V cluster.

Identification of Domains in Cr-TRP1

According to the protein topology prediction method Transmembrane Helices Hidden Markov Model (Krogh et al., 2001), the transmembrane domain in Cr-TRP1, as expected, consists of six transmembrane helices extending from residues 454 to 743 (Figure 3A). A section of this protein comprising over 70% of its length shares 19.2% to 23.3% sequence identity with the transmembrane domains of TRPC1, TRPC7, and several TRPM channels. In order to investigate the presence of domains known to exist in the various TRP functional families (Latorre et al., 2009), we performed a multiple sequence alignment of Cr-TRP1 using the sequences of the 68 functionally characterized TRP channels. Then, a group of subfamily representatives with specific, characterized domains were aligned among themselves plus Cr-TRP1, so that the domain boundaries in Cr-TRP1 could be determined. We identified two ankyrin repeats in the N-terminal region of Cr-TRP1 (Figure 3A; Supplemental Figure 1). While the first ankyrin repeat (a protein-protein interaction motif) has always been observed in TRP channels, the second ankyrin repeat is a novel structural feature.
Figure 1. Cr-TRP1 within the SSN of the TRP Family.

Nodes represent protein sequences, and edges represent the lowest reciprocal BLASTP E-values that exceed a given threshold. Colored nodes correspond to functionally characterized TRP channels, and different shapes are used to identify channels from *C. reinhardtii*, *V. carteri*, and other algae or unicellular organisms.

(A) SSN of 2841 representative sequences with edges filtered to E-value < $1 \times 10^{-18}$, median alignment length of 651 residues, and median identity of 35.8%.

(B) SSN of 7126 sequences with edges filtered to E-value < $1 \times 10^{-87}$, median alignment length of 812 residues, and median identity of 48%.
to 157) is similar in sequence to ankyrin repeats from the TRPA, TRPC, TRPN, and TRPV families, the second ankyrin repeat (residues 166 to 192) is similar in sequence to those from TRPAs, TRPNs, and TRPVs (Latorre et al., 2009). Interestingly, the multiple sequence alignment also shows the presence of two TRPM homology regions (MHRs, stretches of amino acids that share some sequence similarity) similar to MHR2 and MHR4 from TRPM channels (Figure 3A; Supplemental Figure 2). The former extends from residues 82 to 132, and the latter from residues 340 to 440. Therefore, our alignments indicate a small overlap between the end of MHR2 and the beginning of the first ankyrin repeat. The alignments also identified the presence of a partially conserved TRP domain (TD) in the C-terminal region of Cr-TRP1, which extends from residues 757 to 779 (Figure 3B). The TD, an ~25-amino acid motif located immediately after the TM6 transmembrane helix, is a shared feature among channels from the C, M, N, and V sub-families (Latorre et al., 2009). This domain contains the TRP-box, a highly conserved region defined by the consensus sequence WKFQR, in which tryptophan is the most conserved residue. The positive charges at positions 2 and 5 of the sequence are shared by almost all TRPs having a TD and have been suggested to be part of the PIP2 sensor of the channel (Latorre et al., 2009). Although lacking the signature tryptophan in the TRP-box stretch, Cr-TRP1 exhibits an aromatic residue (Phe-758) in that position, together with charged amino acids (Arg-759 and Lys-762, respectively) at equivalent positions to those observed in mammalian TRPMs.
Thus, Cr-TRP1 possesses a TD homologous to that present in all known TRP channels, including highly conserved aromatic and polar residues at the expected positions within the TRP-box. Interestingly, the transmembrane prediction showed a hydrophobic domain at the C terminus of the sequence. The presence of such a hydrophobic stretch in the C-terminal domain of TRPs is not novel and has been associated with the presence of coiled-coil domains in the TRPC and TRPM channels (Wes et al., 1995; Latorre et al., 2009). An alignment of the C-terminal region of Cr-TRP1 with bona fide coiled coils present in the C termini of TRPMs showed low levels of similarity and poor conservation of the heptad repeat. Additionally, none of several algorithms used predicted a coiled-coil domain with high probability.

Recently, TRP channels from the C, M, and V families have been proposed to be mediators of redox sensitivity (Hara et al., 2002; Takahashi and Mori, 2011). Although reactive oxygen species (ROS) may alter TRP channel function through a variety of pathways, including oxidation of channel agonists of a lipidic nature, cysteine residues appear to be good candidates for ROS-dependent modification (Takahashi and Mori, 2011). Several intracellular cysteines located within the ankyrin repeat region and the TD helix have been described (Takahashi and Mori, 2011). Interestingly, Cr-TRP1 has intracellular cysteine residues located in three regions of importance: the ankyrin repeats, the linker connecting TM2 and TM3, and the TD helix. Further mutagenesis studies are necessary to explore ROS-dependent modulation in this novel member of the TRP family.

To summarize, Cr-TRP1 contains a mosaic of features typically segregated in different TRP families (Figure 3A): an N terminus with two ankyrin repeats similar to TRPAs, TRPCs, TRPNs, and (Figure 3B). Thus, Cr-TRP1 possesses a TD homologous to that present in all known TRP channels, including highly conserved aromatic and polar residues at the expected positions within the TRP-box. Interestingly, the transmembrane prediction showed a hydrophobic domain at the C terminus of the sequence. The presence of such a hydrophobic stretch in the C-terminal domain of TRPs is not novel and has been associated with the presence of coiled-coil domains in the TRPC and TRPM channels (Wes et al., 1995; Latorre et al., 2009). An alignment of the C-terminal region of Cr-TRP1 with bona fide coiled coils present in the C termini of TRPMs showed low levels of similarity and poor conservation of the heptad repeat. Additionally, none of several algorithms used predicted a coiled-coil domain with high probability.

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TRPVs and two TRPM homology regions; a transmembrane region similar to TRPCs; and a C terminus containing a TD similar to those in TRPCs, TRPMs, and TRPVs, conserving the positively charged residues common to all TRPMs.

**Cloning, Heterologous Expression, and Biophysical Characterization**

The cloned channel gene (Supplemental Figure 3) was first expressed by transfecting Human Embryonic Kidney (HEK)-293T cells with a green fluorescent protein (GFP)-tagged version. The GFP signal was found near or in the plasma membrane when observed by total internal reflection fluorescence (TIRF) microscopy (Figure 4A); however, intracellular positive signals were also observed. We interpret this as a consequence of overexpression. In order to describe TRP1 channel activity, HEK-293T cells were transfected with the untagged version of the channel. Ionic currents were recorded using whole-cell patch clamp. Current-voltage curves obtained from voltage ramps show a clear outward rectification, similar to currents described previously for mammalian TRPM5 channels (Figure 4B; Supplemental Figure 4) (Hofmann et al., 2003; Clapham, 2009). The nonselective blockers ruthenium red, La3+, and 2-aminoethoxydiphenyl borate and the more TRP-specific blocker BCTC (Valenzano et al., 2003; Clapham, 2009) were tested on transfected HEK-293T cells. TRP1 current was blocked by La3+ (500 μM; Figure 4B), ruthenium red (1 mM; Figure 4B), and BCTC (IC50 = 1.03 μM; Figures 4B and 4C). By contrast, 2-aminoethoxydiphenyl borate

**Figure 4.** Functional Characterization of TRP1 Expressed in HEK-293T Cells.

(A) Transfection of the TRP1-GFP clone in HEK-293T cells. The images present one cell in epifluorescence mode (left) and one cell in TIRF mode (right). Bar = 10 μm.

(B) Current-voltage relations determined from voltage ramps from −100 to +140 mV obtained from HEK-293T cells transfected with TRP1 in pTracer vector (untagged). The curves were obtained under symmetrical Na+ conditions (box; concentrations in mM), and each represents the average of four independent experiments. Statistics are available in Supplemental Figure 4.

(C) Dose-response curve showing the blocking effect of BCTC on the TRP1 conductance (IC50 = 1.03 μM; n = 5). Error bars correspond to se.

(D) Representative curves showing changes in the Vrev (Erev) we used to determine the permeability ratio \( P_{X} / P_{Na} \) when the external solution was replaced. The table above the plot contains the calculated permeability ratios and the corresponding Erev (n = 4).
(50 μM) and the 3,5-bis(trifluoromethyl)pyrazole derivative BTP2 (10 μM; a TRPC channel blocker and activator of TRPM4) were ineffective. To determine the ion selectivity of TRP1, we analyzed shifts in the reversal potential ($V_{\text{rev}}$) after exchanging the bath solution with 140 mM KCl. However, replacement of the bath solution with 140 mM N-methyl-D-glucamine (NMDG+, a large monovalent cation) or Ca$^{2+}$-containing solutions resulted in a shift of $V_{\text{rev}}$ toward negative potentials ($-39$ and $-4.6$ mV, respectively). Therefore, although permeable to Ca$^{2+}$, the channel showed greater preference for monovalent cations (Figure 4D, table), a feature only observed in the calcium-activated channels TRPM4 and TRPM5 (Clapham, 2009). Cr-TRP1 is activated by membrane depolarization, as shown in Figure 5A. After a depolarizing pulse, the instantaneous tail current follows a linear ohmic relationship (Figures 5A and 5B). The observed outward rectification, therefore, must come from a voltage-dependent gating process similar to other voltage-dependent TRP channels including TRPMs and TRPVs (Clapham, 2009). A more precise quantification of open probability and equivalent charge was obtained from the tail currents taken at $-100$ mV (Figure 5C) plotted against activation voltage (Figure 5D). The conductance-voltage ($G-V$) curve was well fit by a Boltzmann distribution with a half-maximal activation voltage ($V_{0.5}$) around $-40$ mV.

The sequence of Cr-TRP1 contains two conserved arginines when compared with mammalian TRPMs (i.e., one located at the top of TM3 and the other located at the bottom of TM4) (Supplemental Figure 5). While the former has not been explored, the latter position has previously been associated with the voltage-dependent gating of TRPM8 channels (Voets et al., 2007) and PIP2 allosteric interactions in temperature-activated TRP channels (Brauchi et al., 2007; Latorre et al., 2009). In TRPV1, the tryptophan at position 697, located within the TRP-box, is critical for the activation of the channel and cannot be replaced by other aromatic residues without severely affecting functional expression (Gregorio-Teruel et al., 2014). Naturally replaced by a phenylalanine in Cr-TRP1’s TRP-box (Figure 3), apparently the role of Phe-758 is well compensated, since strong currents can be observed. Polar amino acids located in the TD helix have been implicated in PIP2-dependent channel modulation (Clapham, 2009; Latorre et al., 2009). As expected, incubation with wortmannin (10 nM), an inhibitor of phosphati-dylinositol 3-kinase, was enough to diminish TRP1 whole-cell currents observed in transfected HEK-293T cells (Supplemental Figure 6). Because the TD helix has been proposed to be a negative allosteric modulator of TRP channel activation (Latorre et al., 2009; Gregorio-Teruel et al., 2014), we tested a subtle mutation within the conserved TRP-box sequence. The position Arg-759 is equivalent to Lys-698 in TRPV1, a residue that was recently suggested to be important for the regulation of the channel’s gate (Steinberg et al., 2014). To test this directly, we replaced the naturally occurring arginine with the conserved lysine residue present in most mammalian channels. The mutant channel now elicited functional characteristics of mammalian channels, with evidently slower activation kinetics of the macroscopic current and the G-V curve shifted $-180$ mV toward more positive potentials ($V_{0.5} = +148$ mV; Figure 5D). This result highlights the important role of the TD helix in the regulation of channel gating and the fine-tuned modulation that occurs in this region (Brauchi et al., 2007; Gregorio-Teruel et al., 2014; Steinberg et al., 2014).

Thus, all our observations are in reasonable agreement with architecture, voltage dependence, and the well-described regulatory role of the C-terminal domain in mammalian TRPM and TRPV channels (Clapham, 2009; Latorre et al., 2009).

A subset of TRP channels, dubbed thermoTRPs, display an exquisite thermal sensitivity, often having $Q_{10}$ values over 10 (Clapham, 2009; Latorre et al., 2009; Pertusa et al., 2012). As several channels from the C, M, and V families have been described as temperature-activated channels (Pertusa et al., 2012), we analyzed Cr-TRP1’s temperature sensitivity. Whole-cell recordings were performed at 14, 22, and 28°C (Figure 5E), producing an evident increase in the macroscopic current (Figures 5E and 5F). It is important to note that although the calculated temperature dependence of the channel allows it to be classified as temperature-dependent ($Q_{10} = 5, n = 5$; Figure 5G), the activation coefficient was modest when compared with the mammalian thermoTRP set ($Q_{10} > 10$; Pertusa et al., 2012).

**DISCUSSION**

We have shown that Cr-TRP1 is a functional TRP channel from algae. Our conclusion that Cr-TRP1 encodes a member of a novel family of polymodal receptors is based on the fact that the channel presents sequence features that are responsible for functional properties typical in TRP channels, such as voltage-dependent outward rectification, cationic nonselective permeability, BCTC blocking, TD-associated modulation, and temperature-dependent gating. Yet, sequence similarity as well as phylogenetic reconstruction indicate that Cr-TRP1 might be different enough to be considered part of a novel family. Additionally, characteristic structural features that are segregated in TRP channels from insects and mammals are present and tied together in this novel TRP clone, which is a relative of channels from the C, M, and N families in the A/C/M/N/V cluster identified by phylogenetic reconstruction. The striking conservation observed for the TD and the TM3-TM4 domain underscores the importance of these regions in the overall structure.

Under physiological conditions of the algae (i.e., low-sodium freshwater), Ca$^{2+}$ and K$^+$ are the major contributors to the voltage changes observed in the *Chlamydomonas* plasma membrane (Harris, 2001; Hegemann, 2008). While light-dependent depolarization is mainly driven by Ca$^{2+}$ and H$^+$ influx, a K$^+$ efflux repolarizes the algal membrane toward the K$^+$ equilibrium potential (Hegemann, 2008). Considering algal sensory physiology and TRP1’s permeability and voltage dependence, it is not unreasonable to speculate that TRP1 channels might function downstream of the sensory input generation (e.g., light-induced Channelrhodopsin activation), contributing to the regeneration of the resting potential after sensory-dependent depolarization. An association between photosensory and chemosensory pathways has been suggested in the past (Govorunova and Sineshchekov, 2005), and there is experimental evidence supporting the idea that the potassium conductance linking these pathways may
Figure 5. Voltage and Temperature Dependence of TRP1 Expressed in HEK-293T Cells.

(A) Instantaneous current protocol for TRP1 (top left). The cell was exposed to a +160-mV depolarizing potential pulse and then pulsed to voltages between –160 and +160 mV in 20-mV increments (bottom left). Macroscopic tail currents were fitted with a single exponential function (dotted line) to obtain the instantaneous currents at zero time (bottom right). The box depicts the ionic concentrations (in mM) in the whole-cell configuration used. The blue and red arrows here and in (B) indicate the steady state and instantaneous currents, respectively.

(B) The steady state curve (closed gray circles, blue arrow) rectifies while the instantaneous current-voltage curve (open orange circles, red arrow) follows an ohmic behavior. Closed black circles correspond to the uncorrected raw data, showing a discrete rectification of the ohmic behavior.

(C) Representative traces obtained in transfected cells subjected to voltage steps from –160 to +200 mV in 20-mV increments. The holding potential was 0 mV, and the tail current was taken at –100 mV. Ionic concentrations (in mM) used in the whole-cell configuration are depicted in the box.

(D) G-V curves were obtained by plotting peak tail currents at –100 mV in response to voltage-activated steady state currents as in Figure 5C (wild type, $z = 1.09, V_{0.5} = –39.3$ mV [$n = 9$]; R759K, $z = 0.8, V_{0.5} = +148$ mV [$n = 5$]).

(E) Representative traces obtained from transiently transfected HEK-293T cells subjected to voltage steps from –100 to +160 mV in 20-mV increments at three different temperatures.

(F) Current-voltage (I-V) relations were obtained by plotting steady state currents depicted in Figure 5E. Each represents the average of five independent experiments.

(G) Current density at different temperatures taken from two fixed voltages (–100 and +100 mV). Error bars correspond to SE.
correspond to a nonelective cation-permeant channel (Nonnengässer et al., 1996). The temperature dependence we describe here might help to define permissive temperatures at which the algae can repolarize, affecting navigation accordingly. Such TRP channel-dependent modulation of the sensory input, downstream of receptor activation, has been proposed before for mammalian TRPM channels such as the taste-associated channel TRPM5 (Liman, 2007) and TRPM1 channels involved in photosensation (Shen et al., 2009). As the canonical role of TRP channels is to serve as cellular sensors (Clapham, 2009), we cannot rule out the possibility that TRP1 might function as a temperature detector in the same way that mechanosensitivity has been associated with *Chlamydomonas* TRP11 channels (Fuju et al., 2011). Further experiments, conducted in algae, will be needed to confirm these hypotheses. We are aware that, without experimental evidence of the subcellular localization of TRP1, it is difficult to speculate further on its role. However, previous reports demonstrated that delflagellation upregulates TRP1 expression, suggesting a flagellar localization (Fuju et al., 2011).

Action potentials observed in animal cells are generally based on Na⁺ and/or Ca²⁺ conductances. However, plants lack genes coding for important mediators found in the animal sensory response, such as voltage-dependent Ca²⁺ channels (Ca²⁺v), TRP channels, inositol 1,4,5-trisphosphate receptors, and ryanodine receptors (Wheeler and Brownlee, 2008). Interestingly, chlorophyte algae such as *Chlamydomonas* use Ca²⁺v channels in signaling. Therefore, Ca²⁺v-dependent signaling mechanisms, while absent in land plants, are present in these single-celled eukaryotes. The widespread distribution of these channels in modern, extant organisms suggests that they were present in a common ancestor of plants and animals; the most recent common ancestor existed ~1.6 billion years ago (Nei et al., 2001; Yoon et al., 2004). In the metazoan lineage, ion channel diversification can be traced down to choanoflagellates, a group of free-living unicellular and colonial flagellate eukaryotes considered to be the closest living relatives of animals, where few putative TRP channel genes have been reported (Martincic et al., 2008; Jegla et al., 2009). The presence of a functional TRP channel in *Chlamydomonas* implies a deep evolutionary origin of this gene family. Notably, the conservation of structural-functional features through more than 1.5 billion years of evolution underscores the robustness within this diverse family of ion channels (Latorre et al., 2009; Jegla et al., 2009).

We envision that Cr-TRP1 was inherited from a common ancestor of plants and animals; therefore, the precursor of modern TRP channels must have been present in a unicellular organism predating the appearance of *Chlamydomonas*. In fact, several other single-celled organisms appear to possess sequences encoding TRP channels, including *Dictyostelium*, *Trypanosoma*, *Leishmania*, and *Plasmodium* (Martincic et al., 2008), yet, according to our results, these channels should belong to the evolutionarily distinct P/ML cluster of TRP proteins.

### METHODS

**Identification of TRP-Like Channels from Green Algae and Unicellular Organisms**

Protein sequences corresponding to the 67 TRP ion channels functionally annotated in the International Union of Basic and Clinical Pharmacology database (http://www.iuphar-db.org/DATABASE/FamilyMenuForward?familyId=78), together with NompC, were downloaded. The data set contains representatives of families TRPA, TRPC, TRPM, TRPML, TRPN, TRPP, and TRPV. These 68 functionally characterized TRP channels were clustered at 50% sequence identity using CD-HIT (Li and Godzik, 2006), resulting in 23 clusters. A representative from each cluster was then used as a query for BLASTP searches (BLAST 2.2.28+; Altschul et al., 1990) against the proteome of *Chlamydomonas reinhardtii* downloaded from Phytozome version 9.0 (Goodstein et al., 2012). From the output, we chose proteins that presented BLASTP E-values > 1e⁻⁶ with at least two of the representative sequences. Then, we performed a new BLASTP search, this time using just the transmembrane regions of the 68 known TRP ion channels against the putative TRPs returned from our previous search. We identified 10 gene products that aligned well (alignment length > 70% of sequence length) with the transmembrane region of at least one TRP ion channel in our data set. The procedure was automated in a pipeline involving Python scripts and MySQL searches and was used to identify TRP subgroup members in the proteomes of *Coccomyxa subellipsoidea C-169* (Cs), *Micromonas pusilla* CCMP1545 (Mp_COMP), *Micromonas pusilla* RCC299 (Mp_RCC), *Ostreococcus lucimarinus* (Ol), and *Volvox carteri* (Vc) downloaded from Phytozome. We also downloaded from UniProt the complete proteomes of species from the following genera, *Paramecium*, *Dictyostelium*, *Trypanosoma*, *Leishmania*, and *Plasmodium*, totaling 17 complete proteomes: *Paramecium tetraurelia* (Pt), *Dictyostelium discoideum* (Dd), *Dictyostelium purpureum* (Dp), *Trypanosoma brucei brucei* (strain 927/4 GUTat10.1), *Trypanosoma brucei gambiense* (strain MHOM/CI/86/DAL972), *Trypanosoma cruzi* (strain CL Brener; TcCL), *Trypanosoma cruzi* (Tc), *Leishmania braziliensis*, *Leishmania infantum* (Lj), *Leishmania major* (Lm), *Leishmania mexicana* (strain MHOM/GT/2001/U1103; Lmx), *Plasmodium berghei* (strain Anka), *Plasmodium chabaudi*, *Plasmodium falciiparum* (isolate 3D7), *Plasmodium knowlesi* (strain H), *Plasmodium vivax* (strain Salvador I), and *Plasmodium yoelii* yoelii. In total, 33 putative TRP channels were found among algae and unicellular organisms (Figure 2).

**SSN of the TRP Family**

To generate the SSN, we started from the set of 68 functionally characterized TRP ion channels (seed sequences) and aimed at identifying all potential TRP family members present in NCBI’s nr database. To avoid redundancy in the searches, a representative from each of the 23 clusters described previously was used to perform BLASTP searches against a local nr database downloaded from the NCBI ftp site on April 29, 2014. Only proteins having hits with BLASTP E-values > 1e⁻⁶ to at least two of the representative sequences were considered further. This gave us an initial set of 38,263 sequences. As described before, the transmembrane regions of the 68 seed sequences were used to perform searches against the 38,263 putative TRPs returned from our previous search, and only those 7107 sequences that aligned with at least 70% of the full length of the transmembrane domain of at least one representative were considered further. All 7107 sequences plus the 33 putative TRP channels from algae and unicellular organisms were used to generate an SSN, where nodes correspond to sequences and edges to BLASTP E-values between the sequences. We note that 17 of the 33 putative TRP channels were already included in the nr data set, so the total number of proteins in the network is 7126. Because the SSN generated had too many edges (over 8 million), an SSN of representative sequences from the family was also created. For this SSN, the 7126 unique TRP subgroup members were clustered at 90% sequence identity using CD-HIT, resulting in 2841 clusters. The largest sequence in each cluster was used as a representative for generating an SSN. Since BLASTP E-values are not symmetrical, we performed searches using one of the sequences under comparison as query and the other as subject, replaced the query with the subject and vice versa, and selected the worst reciprocal BLASTP E-value for each pair. Visualization was performed using the organic layout in Cytoscape 2.8.3 (Cline et al., 2007) at different thresholds for the E-value. The two E-values selected for generating Figure 1 correspond as follows: Figure 1A,
the highest E-value where cluster A/C/M/N/V is separated from cluster P/ML
(1e-18). Figure 1B, the highest E-value where the seven functional families are
separated from one another (1e-8).

Phylogenetic Inference for the TRP Family
A multiple sequence alignment for the 68 functionally characterized TRPs plus
the 33 sequences identified in algae and unicellular organisms was built in
MAFFT (Katoh et al., 2002) using an iterative refinement method considering both
weighted sums of pairs and consistency scores (Supplemental Data Set 1).
Molecular phylogeny was inferred using the Bayesian method im-
plemented in MrBayes version 3.2 (Ronquist and Huelsenbeck, 2003). To
estimate the best fixed-rate model for our data, we used a mixed model,
obtaining a posterior probability of 1 for the Jones model (Jones et al., 1992).
Thus, the molecular phylogeny presented here uses the Jones substitution
model and a gamma distribution model to allow rate variation among sites.
We set the number of generations to 2 x 10^6, sampled every 1000th gen-
eration, and discarded the initial 30% of samples before summarizing trees
and parameters. The average so of split frequencies was less than 0.0026
upon convergence.

Identification of Domains in TRP1
Transmembrane Helices Hidden Markov Model was used to define transmembrane segments. All other domains were defined from the literature
(Latorre et al., 2009) and identified in multiple sequence alignments of bona fide
TRP channels against CR-TRP1 performed in PROMALS3D (Pei and Grishin,
2014). For clarity, the alignments presented here incorporate only one channel
per subfamily member. The protein illustration for CR-TRP1 that includes the
identified domains was created using Proter (Omasits et al., 2014).

Cell Culture
Wild-type C. reinhardtii strain CC-124 mt− was maintained in Sueoka’s
medium (Harris, 2001) at 18°C under constant agitation and light/dark pe-
riods of 12 h. C. reinhardtii gametes were prepared by incubating vegetative
cells (ATCC). Gigaseals were formed by using 2- to 4-M
borosilicate
V

Series resistance and cell capacitance were analogically compensated
corrected for CsF/NaCl solutions. Seal resistance was >2 G

between

TRPC1_HUMAN, NP_003295.1; TRPC1_MOUSE, NP_035773.1;
TRPC2_RAT, NP_057742.2; TRPC2_MOUSE, NP_035774.2; TRPC3_RAT,
Q9R283-1; TRPC3_HUMAN, NP_001124170.1; TRPC3_MOUSE, NP_052383.2;
TRPC4_RAT, NP_068593.2; TRPC4_HUMAN, NP_057263.1; TRPC4_MOUSE,
NP_059860.1; TRPC4_RAT, NP_536321.1; TRPCS_HUMAN, NP_036603.1;
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Supplemental Data

Supplemental Figure 1. Multiple Sequence Alignment Depicting the Ankyrin Domains Present in Cr-TRP1 and Other TRPN, TRPC, TRPV, and TRPA Family Representatives.

Supplemental Figure 2. TRPM Homology Regions (MHR) in Cr-TRP1.

Supplemental Figure 3. Cr-TRP1 Amino Acid Sequence.

Supplemental Figure 4. TRP1 Homology Regions (MHR) in Cr-TRP1.

Supplemental Figure 5. Alignment at the TM3-TM4 Region.

Supplemental Figure 6. Inward and Outward Whole-Cell Currents Are Affected after 10 min Incubation with 10 nM Wortmannin.

Supplemental Data Set 1. Fasta Format Multiple Sequence Alignment of the 68 Functionally Characterized TRPs Plus the 33 Sequences Identified in Algae and Uncellular Organisms Used to Construct Phylogeny Shown in Figure 2.

ACKNOWLEDGMENTS

We thank H. Kurata, R. Latore, and C. Zunker for commenting on the article and E. Harris for providing strains and helpful information. This work was supported by FONDECYT (Grants 11070190 and 1110906 to S.B., Grant 1131064 to R.M., and Grant 3140233 to C.K.C.), CONICYT (Grant ACT-1113 to D.E. and fellowship to L.A.-D.), and the Millennium Initiative (CINV Grant P09-022-F to D.E.A.).

AUTHOR CONTRIBUTIONS


Received September 8, 2014; revised November 28, 2014; accepted December 21, 2014; published January 16, 2015.

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A Transient Receptor Potential Ion Channel in *Chlamydomonas* Shares Key Features with Sensory Transduction-Associated TRP Channels in Mammals


*Plant Cell*; originally published online January 16, 2015;
DOI 10.1105/tpc.114.131862

This information is current as of July 6, 2017

Supplemental Data /content/suppl/2015/01/12/tpc.114.131862.DC1.html


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