Polymodal TRPC signaling
Emerging role in phenotype switching and tissue remodeling

Klaus Groschner
Institute of Pharmaceutical Sciences—Pharmacology and Toxicology; University of Graz; Graz, Austria

Key words: transient receptor potential channel 4, endothelial phenotype switching, Ca\(^{2+}\) signaling, cell-cell contacts

TRPC proteins have been implicated in a large array of Ca\(^{2+}\) signaling processes and are considered as pore-forming subunits of unique polymodal channel sensors. The mechanisms of TRPC activation are so far incompletely understood but appear to involve a concert of signals that are generated typically downstream of receptor-mediated activation of phospholipase C. Specifically for the TRPC1/4/5 subfamily the activating scenario is ill-defined and appears enigmatic due to the observation of multiple modes of activation. TRPC4 was initially described as a store-operated cation channel and was repeatedly proposed as a pivotal element of the store-operated signaling pathways of various tissues. However, classical reconstitution of TRPC4 complexes in expression systems as well as recent knock-down strategies provided evidence against store-dependent regulation of this channel and raised considerable doubt in its proposed prominent role agonist-induced Ca\(^{2+}\) signaling. Recent analysis of the function of TRPC4 in vascular endothelial cells of divergent phenotype revealed a novel aspect of TRPC signaling, extending the current concept of TRPC regulation by a phenotype-dependent switch between Ca\(^{2+}\) transport and a potential intracellular scaffold function of the TRPC protein.

The Enigmatic Gating Control of TRPC Channels

All seven members of the “classical” family of transient receptor potential (TRP) proteins are considered as pore forming subunits of non-selective cation channels, which are controlled by cellular phospholipase C (PLC) activity (reviewed in ref. 1–4). Although the key role of PLC in channel activation is without controversy, the molecular mechanisms that govern TRPC channel gating downstream of PLC are in large part elusive. Historically, the TRPC subfamily received substantial interest immediately after identification of the mammalian trp genes, based on their potential function as sensors for depletion of intracellular Ca\(^{2+}\) stores, and most members have been implicated in store-operated Ca\(^{2+}\) entry phenomena in various tissues.\(^6\)–\(^10\) However, an initial, detailed analysis of the channel properties in heterologous expression systems strongly suggested that upon heterologous overexpression in classical host cells such as HEK293 or CHO, most TRPC species form receptor-regulated cation channels that are activated independently of the filling state of the endoplasmic reticulum (ER).\(^11\)–\(^14\) For a subset of TRPC proteins (TRPC3/6 and 7), diacylglycerol was identified as an activating mediator that links these channels to phospholipase C activity.\(^15\) Nonethelss, regulatory communication and even physical interaction with components of the ER has repeatedly been demonstrated for TRPC proteins including also TRPC3/6/7.\(^15\)–\(^22\) Moreover, the potential importance of subunit heteromerization and the formation of signal complexes of certain stoichiometry was found to determine biophysical as well as regulatory properties of TRPC channels.\(^12\)–\(^25\) A particular tight relation between channel activity and the Ca\(^{2+}\) content of the ER appear to exist for the TRPC1/4/5 subset of closer relatives. The more recently identified ER Ca\(^{2+}\) sensor Stim1, which is a key component of store-depletion activated Ca\(^{2+}\) entry via Orai channels, has been demonstrated to interact also with TRPC1/4 and 5 proteins and may confer store-dependent regulation of certain TRPC channel multimers.\(^26\)–\(^28\) Although several gating determinants, key factors and potential mechanisms of channel activation have been reported for TRPC1/4/5 channels, the molecular chain of events leading to activation is incompletely understood. Processes potentially involved in TRPC4/5 channel activation include the targeting of channels into specific membrane domains and interaction with the actin cytoskeleton mediated by ERM-domain proteins,\(^29\)–\(^30\) changes in local PIP\(_2\) content of the channels membrane environment and recruitment and activation of pertussis toxin sensitive G proteins.\(^31\) Moreover local changes in free Ca\(^{2+}\) and/or diveralent concentrations at the cytoplasmic face of the channel complex are a likely determinant of channel gating.\(^32\) Besides integration of lipid, Ca\(^{2+}\) and G-protein signals by the TRPC4 gating machinery, additional impact of physical coupling of TRPC4 channels to ER Ca\(^{2+}\) stores via electrostatic interaction with Stim1 needs consideration.\(^27\) Similar to other TRP channels it appears feasible to speculate that multiple modes of TRPC4 activation might exist, depending on the composition, stoichiometry and cellular localization of the signalplex.

*Correspondence to: Klaus Groschner; Email: klaus.groschner@uni-graz.at
Submitted: 04/21/10; Accepted: 04/21/10
Previously published online:
www.landesbioscience.com/journals/cib/article/12131
DOI: 10.4161/cib.3.5.12131
Plasticity of Signalplexes as the Basis of Polymodal TRPC Function

Variations in TRPC signaling complex composition and stoichiometry have been suspected to underly the divergent regulatory properties of TRPC channels observed along with variation of expression levels and cell types. TRPC4 is for a large part sequestered in intracellular compartments and unavailable for Ca\(^{2+}\) signaling in single cells (contact deficient; upper left). By contrast, formation of immature cell adhesions promotes surface targeting of β-catenin-TRPC4 complexes and enables further recruitment of channels into the plasma membrane and Ca\(^{2+}\) entry function (immature contact; upper right). Once mature barriers are formed (mature contact; lower), TRPC4 resides for a large part in junctional complexes that are rapidly retrieved from the cell surface during growth factor stimulation and are barely available for contribution to global Ca\(^{2+}\) signaling.

TRPC4, a Contested Player in Vascular Endothelial (Patho)physiology

Evidence from a murine TRPC4 knock-down model suggested that this channel protein is of particular importance in the cardiovascular system, representing a prominent endothelial TRPC species. Consequently, the role of TRPC4 in endothelial physiology and pathophysiology has been analyzed extensively using a variety of approaches including siRNA as well as dominant negative knock-down strategies. These investigations yielded highly controversial results ranging from evidence for a key role of TRPC4 in the endothelial store-operated pathway, which controls essential functions such as barrier stability, gene expression and mediator production, to a complete disqualification of TRPC4 as an endothelial Ca\(^{2+}\) transport system. Interestingly, despite the latter report caused serious doubt in a prominent Ca\(^{2+}\) signaling function of TRPC4 in the endothelium, the relevance of this protein as a determinant of endothelial proliferation was further confirmed.

A Phenotype Window Discloses TRPC4 Channel Function in Vascular Endothelium

Recent mistrust in the involvement of TRPC4 in endothelial Ca\(^{2+}\) signaling was based on a convincing demonstration that store-operating Ca\(^{2+}\) entry into endothelial cells is mediated by the Stim1-Orai pathway. Hence, the cellular role of vascular TRPCs became again misty with the glimpse of a possible ion transport independent function of these proteins. At the same time, we investigated agonist-induced changes in plasma membrane expression of endothelial TRPC4 proteins and observed a striking phenotype dependence of surface expression of the channel protein. Most importantly, we failed to detect significant plasma membrane targeting of TRPC4 in single migrating endothelial cells. As this cellular state is typically the substrate for investigation of whole cell membrane conductances and also Ca\(^{2+}\) entry, our observation of unavailable TRPC4 membrane targeting was perfectly in line with the reported lack of Ca\(^{2+}\) signaling function. Nonetheless, proliferating, sub-confluent populations displayed a marked recruitment of TRPC4 into the plasma membrane upon stimulation with either EGF or thrombin, and we identified a TRPC4-mediated Ca\(^{2+}\) signaling pathway specifically in proliferating clusters of endothelial cells that formed immature cell-cell contacts (Fig. 1). TRPC4 expression was without significant impact on Ca\(^{2+}\) signaling in single cells as well as in mature endothelial barriers. In conclusion, TRPC4 appears to function as a Ca\(^{2+}\) entry channel exclusively at a certain cellular state during endothelial development and/or barrier repair/regeneration. Close inspection of phenotype transition and detailed analysis of a rather small phenotype window enabled us to verify and characterize Ca\(^{2+}\) transport function of TRPC4 in native endothelial cells. The transient function of TRPC4 as part of a plasma membrane Ca\(^{2+}\) channel complex raises the question if TRPC4 and other TRPC proteins are able to adopt specific cellular functions when targeted to intracellular membranes and protein complexes. A respective hypothesis was put forward by our finding that TRPC4 associates with the junctional protein β-catenin. This newly identified interaction partner represents another multifunctional signaling molecule that binds to TRPC4 also within the cell in nucleus associated compartments. Thus, TRPC4 may serve endothelial phenotype switching in a highly polymodal manner including intracellular crosstalk with other signaling pathways.
Perspectives and Outlook

The TRPC channel family is considered as a paradigm of multifunctional signaling molecules, based on their polypep- 
dal gating behavior and their typical promiscuity with respect to gating stimuli. The recently uncovered highly phenotype- 
dependent cellular localization and function of TRPC4 adds a new dimension to the concept of polypeptide signaling. The observed intracellular targeting and integration into specific endothelial signalplexes including association and cotranslocation with β-catenin, opens the view on Ca2+-transport independent functions of TRPC proteins and cross-talk with important processes controlling gene expression such as the Wnt pathway. In aggregate, polypeptide TRPC signaling appears to play a crucial role in phenotype transition within the vascular system. The potential role of TRPCs in tissue remodeling and repair needs particular attention and warrant further investigations.

Acknowledgements

I wish to thanks Dr. Michael Potser for helpful discussions and FWF (P19820) as well as ÖNB (8216) for financial support.

References

1. Venkatachalam K, Montell C. TRP channels. Annu Rev Biochem 2007; 76:387-417.
2. Vazquez G, Wedel BJ, Aziz O, Trehbak M, Putney JW Jr. The mammalian TRPC cation channels. Biochim Biophys Acta 2004; 17/42:21-36.
3. Pedersen SF, Owusu-Ansah G, Nilius B. TRP channels: An overview. Cell Calcium 2005; 38:233-52.
4. Birnbaumer L. The TRPC class of ion channels: a critical review of their roles in slow, sustained increases in intracellular Ca(2+) concentrations. Annu Rev Pharmacol Toxicol 2009; 49:395-425.
5. Zhu X, Jiang M, Peyton M, Boulag Y, Huret R, Stefani E, et al. Tip, a novel mammalian gene family essential for agonist-activated capacitative Ca2+ entry. Cell 1996; 85:609-771.
6. Liu X, Wang S, Singh BB, Lockwood T, Jadhav J, O’Connell J, et al. Tip1, a multifunctional polypeptide for the store-operated Ca(2+) influx mechanism in salivary gland cells. J Biol Chem 2000; 275:3403-11.
7. Ng LC, Gurney AM. Store-operated channels mediate Ca(2+)-influx and contraction in rat pulmonary artery. Circ Res 2001; 89:923-9.
8. Grocshner K, Hingel S, Lintschinger B, Bailer M, Romanin C, Zhu X, et al. Tip proteins form store-operated cation channels in human vascular endothelial cells. FEBS Lett 1998; 437:101-6.
9. Freichel M, Suh SH, Pfeifer A, Schwarz W, Tröst C, Weingeberer B, et al. Lack of an endothelial store-operated Ca2+ current impairs agonist-dependent vasodilation in TRP4-/- mice. Nat Cell Biol 2003; 5:1217-24.
10. Philipp S, Tröst C, Warnat J, Rautmann J, Himmerkus N, Schroth G, et al. ETX1, a CCCH-type C-terminal domain protein, is a functionally relevant regulatory polypeptide for TRPC4, a novel redox-sensitive cation channel-evidence for expression in native trpc3/trpc4 heteromeric channels in endothelial cells. J Biol Chem 2006; 281:13588-95.
11. Hofmann T, Obukhov AG, Schaefer M, Harteneck C, Gurney AM. TRPC4 mediates store-operated Ca2+ entry in endothelial cells. Proc Natl Acad Sci USA 2008; 105:2895-900.
12. Kiselevy K, Xu X, Mochayeva G, KuÊ T, Pethaï A, Mignery G, et al. Functional interaction between InsP3 receptor and store-operated htrp3 channels. Nature 1998; 396:478-82.
13. Kiselevy K, Mignery GA, Zhu MX, Mueller S. The N-terminal domain of the IP3 receptor gates store-operated htrp3 channels. Mol Cell 1999; 4:423-9.
14. Huang EJN, Zhang W, KuÊ T, Yen JY, Han A, Mueller S, et al. STIM1 carboxyl-terminus activates native SFC and TRPC4 channels. Nat Cell Biol 2006; 8:1003-10.
15. Woo JS, Kim do H, Allen PD, Lee EH. TRPC3-interacting triad proteins in skeletal muscle. Biochem J 2008; 411:399-405.
16. Strubing C, Krapivinsky G, Krapivinsky L, Clapham DE. TRPC1 and TRPC3 form a novel cation channel in mammalian brain. Neuron 2001; 29:645-55.
17. Strubing C, Krapivinsky G, Krapivinsky L, Clapham DE. Formation of novel TRPC channels by complex subunit interactions in embryonic brain. J Biol Chem 2003; 278:9014-9.
18. Potser M, Graziani A, Rosker C, Eder P, Deleer I, Kahr H, et al. TRPC3 and TRPC4 associate to form a redox-sensitive cation channel-evidence for expression of native trpc3/trpc4 heteromeric channels in endothelial cells. J Biol Chem 2006; 281:13588-95.
19. Yuan JP, Zeng W, Huang GN, Worley PF, Mueller S. TRIM1 heteromultimerizes TRPC channels to determine their function as store-operated channels. Nat Cell Biol 2007; 9:536-45.
20. Zeng W, Yuan JP, Kim MS, Choi HY, Huang GN, Worley PF, et al. STIM1 gates TRPC channels, but not Orai1, by electrostatic interaction. Mol Cell 2008; 32:439-48.
21. Cheng KT, Liu X, Ong HL, Ambudkar IS. Functional requirement for Orai1 in store-operated TRPC1-StIM1 channels. J Biol Chem 2008; 283:12395-40.
22. Mery L, Snauss B, Dufoi JE, Krause KH, Hoth M. The PDZ-interacting domain of TRPC4 controls its intracellular targeting and surface expression in HEK293 cells. J Cell Sci 2002; 115:3497-508.
23. Obukhov AG, Nowycky MC. TRPC5 activation kinet- ics are modulated by the scaffolding protein ezrin/radixin/moesin-binding phosphoprotein-50 (EBP50). J Cell Physiol 2004; 201:227-35.
24. Otsuguro K, Tan J, Tang Y, Xiao R, Freichel M, Tsvolovskyy V, et al. Isomform-specific inhibition of TRPC4 channel by phosphatidylinositol 4,5-bisphospho- late. J Biol Chem 2008; 283:10026-36.
25. Blair NT, Kacmarcik JS, Clapham DE. Intracellular calcium strongly potentiates agonist-activated TRPC5 channels. J Gen Physiol 2009; 135:525-46.
26. Trebak M, Bird GS, McKay RR, Putney JW Jr. Comparison of human TRPC3 channels in recep- tor-activated and store-operated modes. Differential sensitivity to channel blockers suggests fundamental differences in channel composition. J Biol Chem 2002; 277:21671-23.
27. Bergdahl A, Gnozzi MF, Willhöft AK, Erzinge L, Eyjolfson A, Xu SZ, et al. Plasticity of TRPC expression in arterial smooth muscle: correlation with store-operated Ca2+ entry. Am J Physiol Cell Physiol 2005; 288:C72-80.
28. Trebak M, Vaqueiro G, Bird GS, Putney JW Jr. The TRPC3/6/7 subfamily of cation channels. Cell Calcium 2003; 33:451-61.
29. Gouchner K, Rosker C, TRPC3: A versatile transducer molecule that serves integration and diversification of effector signaling. Naunyn Schmiedeberg Arch Pharmacol 2005; 371:251-6.
30. Liao Y, Plummer NW, George MD, Abramowitz J, Zhu MX, Birnbaumer L. A role for Orai in TRPC-mediated Ca2+ entry suggests that a TRPC-Orai complex may mediate store and receptor operated Ca2+ entry. Proc Natl Acad Sci USA 2009; 106:3202-6.
31. Eder P, Gouchner K TRPC3/6/7: Topical aspects of biophysics and pathophysiology. Channels 2008; 2:94-9.
32. Freichel M, Vennekens R, Olausson J, Hoffmann M, Muller C, Stolz S, et al. Functional role of TRPC proteins in vaso lesions from TRPC-deficient mouse mod- els. Biochem Biophys Res Commun 2004; 322:1532-8.
33. Ciolfi DL, Stevens T. Regulation of endothelial cell barrier function by store-operated calcium entry. Microcirculation 2006; 13:709-25.
34. Tiruppathi C, Freichel M, Vogel SM, Paria BC, Mehra D, Flockerzi V, et al. Impairment of store-operated Ca2+ entry in TRPC4(-/-) mice interferes with increase in lung microvascular permeability. Circ Res 2002; 91:70-6.
35. Tiruppathi C, Minshall RD, Paria BC, Vogel SM, Malik AB. Role of Ca2+ signaling in the regulation of endothelial permeability. Vascul Pharmacol 2002; 39:173-85.
36. Abdullayev IF, Bisalillom JM, Potier M, Gonzalez JC, Motiani RK, Trebak M. Stl1 and Orai1 mediate CRAC currents and store-operated calcium entry important for endothelial cell proliferation. Circ Res 2008; 103:1289-99.
37. Graziani A, Potser M, Huepel WM, Schleifer H, Krenn M, Drenckhahn D, et al. Cell-cell contact formation governs Ca2+ signaling by TRPC4 in the vascular endothelium. Evidence for a regulatory TRPC4-beta-catenin interaction. J Biol Chem 2009; 285:4213-25.