Cell Calcium 67 (2017) 91–104

Contents lists available at ScienceDirect

Cell Calcium

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

Review

Genetic strategies to analyze primary TRP channel-expressing cells in mice

Amanda Wyatt[a,1], Philipp Wartenberg[a,1], Michael Candlish[a], Gabriela Krasteva-Christ[b], Veit Flockerzi[a], Ulrich Boehm[a,*]

[a] Pharmacology and Toxicology, Center for Molecular Signaling (PZMS), Saarland University School of Medicine, Homburg, Germany
[b] Institute of Anatomy and Cell Biology, Saarland University School of Medicine, Homburg, Germany

ARTICLE INFO

Article history:
Received 10 April 2017
Received in revised form 15 May 2017
Accepted 15 May 2017
Available online 17 May 2017

Keywords:
TRP channels
TRPM5
Gene targeting
Homologous recombination
Knock-in
Genetic labeling
Internal ribosomal entry site
Cre recombinase
ROSA26
τGFP

ABSTRACT

Transient receptor potential (TRP) ion channels regulate fundamental biological processes throughout the body. TRP channel dysfunction has been causally linked to a number of disease states and thus establishes these channels as promising therapeutic targets. In order to dissect the physiological role of individual TRP channels in specific tissues, a detailed understanding of the expression pattern of the different TRP channels throughout the organism is essential. We provide an overview of recent efforts to generate novel TRP channel reporter mouse strains for all 28 TRP channels encoded in the mouse genome to understand expression of these channels with a single-cell resolution in an organism-wide manner. The reporter mice will enable both the visualization and manipulation of all primary TRP channel-expressing cells allowing an unprecedented wealth in variety to investigate TRP channel function *in vivo*. As proof of principle, we provide preliminary results documenting TRPM5 expression throughout the entire body of juvenile and adult mice.

Contents

* Corresponding author at: Experimental Pharmacology, Center for Molecular Signaling (PZMS), Saarland University School of Medicine, Bldg 61.4, 66421 Homburg, Germany.
E-mail address: ulrich.boehm@uks.eu (U. Boehm).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ceca.2017.05.009
0143-4160/

1. Mouse models for TRP channelopathies

TRP channels function as cellular sensors [1] that are required to respond to changes in a wide variety of cellular environments through mediating numerous sensory signals. The 28 TRP channels encoded in the mammalian genome can be divided into six subtypes, namely TRPM (Melanostatin), TRPP (Polycystin), TRPC (Canonical), TRPML (Mucolipin), TRPV (Vanilloid) and TRPA (Ankyrin) [2]. Expression patterns of the different TRP channels vary widely between family members with channels such as TRPV1 and TRPM8 showing narrowly tuned expression mostly restricted to sensory neurons [3] while TRPM7 is for example expressed to a high level almost ubiquitously [4]. TRP channels are composed of six transmembrane spanning domains with the loop between the fifth and sixth forming a cation-permeable pore [5–9]. TRP channel regulation of fundamental biological processes underpinning a plethora of functions throughout the body is reflected by the many 'TRP channelopathies' [10] observed as a result of dysfunction of these channels. In humans, mutations in the gene for *TRPP2* (synonym polycystic kidney disease 2 or *PKD2*) for example have been identified which result in polycystic kidney disease; in which large fluid-filled cysts form in the kidney destroying normal renal function and leading to end-stage renal disease in 50% of cases [11,12]. Similar disease phenotypes are also seen in mouse models lacking functional PKD1 [13], which is not a TRP channel.

Mutations in the gene encoding *TRPML1* cause the neurodegenerative lysosomal storage disorder mucolipidosis type IV [14]. This disease encompasses a large range of symptoms including psychomotor retardation, blood iron deficiency, ophthalmologic abnormalities and agenesis of the corpus callosum [15].

TRPM6 dysfunction has been associated with hypomagnesemia with secondary hypocalcemia, a disease in which intestinal Mg^{2+} absorption is impaired in addition to renal Mg^{2+} leak [16,17]. These together result in very low Mg^{2+} and Ca^{2+} serum concentrations and present as neurological symptoms such as muscle spasms and seizures in infants, and death if left untreated.

The wide-ranging physiological importance of TRP channels establishes these as potentially appealing therapeutic targets [18]. In contrast to other families of ion channels where subtype-specific targeting can be complicated due to a high level of homology between members, TRP channels are more dissimilar to each other and may therefore allow pharmacological subtype distinction [19]. In order to facilitate this, however, a detailed understanding of the expression pattern of the individual TRP channels throughout the organism is first required. The generation of TRP channel reporter mouse strains will help to understand expression of these channels in an organism-wide manner with a single-cell resolution. This should then help to predict and control various unwanted effects that may occur and possibly lead to more targeted ion channel therapies.

1.1. Conditional TRP channel-deficient mouse models

So far, half of the published TRP-deficient mice are conventional or global knockout (KO) mice (for a list of available mouse strains see [20]). The gene deletion is unrestricted and animals inherit the recombinant allele in all of their cell types. In these animals, it may be difficult to exclude the possibility that developmental defects or compensatory up-regulation of other genes contribute to the phenotype observed in adult animals. In addition, this global gene deletion might make it difficult to attribute abnormal phenotypes to a particular type of cell. Conditional KOs allow regional and temporal control of *Trp* gene expression [21–28] and restrict deletions to cells in a specific tissue or at specific points in an animal's development. More conditional TRP alleles are certainly required to unravel TRP channel function in a given cell type. The generation of additional conditional TRP KO alleles such as for *Trpc1* is in progress, following a well-established protocol utilized to generate, for example, TRPM4 [29], TRPV6 [30,31] and TRPC5 [32] conditional KOs.

2. Binary genetic strategies

2.1. The TRP-IRES-Cre zoo

To understand the function of TRP channels in body homeostasis, it is of pivotal importance to study primary TRP channel-expressing cells rather than tissue culture cells transfected with individual TRP channels. Despite its importance, information about primary TRP channel-expressing cells is still sparse. This is at least partially due to the difficulty to generate reliable antisera against TRP channels that can be used in immunohistochemistry to identify and characterize primary TRP channel-expressing cells *in situ* and *in vivo*. One promising experimental approach to make these cells amenable for experimental analysis is genetic labeling in mice. Because information about the promoter/enhancer regions of most *Trp* genes is rather limited, we use gene targeting (instead of a conventional transgenic approach). First, because Cre-mediated recombination is irreversible, labeled cells in these animals accurately reflect the history of promotor activity of the individual *Trp* gene. Second, many Cre-dependent reporter/effector strains have been generated and are readily available allowing an unprecedented wealth of possibilities to experimentally interrogate TRP channel-expressing cells in mice (Fig. 1).

To be able to visualize and manipulate TRP channel-expressing cells at a single cell resolution, we generate mice in which Cre recombinase is co-expressed with individual *Trp* genes. Using gene targeting in embryonic stem cells, the respective *Trp* genes are modified by inserting an internal ribosome entry site (IRES) sequence followed by a *Cre recombinase* complementary DNA. The altered stem cells are then used to generate TRP-IRES-Cre "knock-in" mice (Fig. 2). To remove the neomycin resistance cassette, TRP-IRES-Cre^{neo+} animals are bred to Flp recombinase deleter mice [33].

To generate these mice, an internal ribosome entry site (IRES) followed by a Cre recombinase cDNA is inserted 3′ to the stop codon of the respective TRP gene. By using an IRES, bicistronic expression of the TRP channel and Cre-recombinase is ensured, however with the caveat that the coding sequence 3′ of the IRES sequence is often expressed at significantly lower levels than that of the coding sequence 5′ of the IRES [34]. One alternative approach would be to insert a 2A "self-cleavable" peptide sequence. This approach results in stoichiometric expression of both the upstream and downstream proteins. However, the generation of fusion proteins has been reported using this strategy [35] and therefore has the potential to inadvertently result in physiological aberrations. By utilizing an IRES, the expression of the upstream protein should be unaffected [34] and prevent the generation of undesirable fusion proteins [35]. As TRP channels are typically expressed at very low