

# TRPC3-dependent synaptic transmission in central mammalian neurons

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**Abstract** The transient receptor potential (TRPC) proteins form non-selective cation channels that are activated downstream of G<sub>q</sub>-phospholipase C-coupled receptors. TRPC3, one of the seven members of the TRPC subfamily, combines functions of an unspecific ion channel and a signal transducer. In the mammalian brain, the expression of TRPC3 is highest in cerebellar Purkinje cells, the principal neurons, and the sole output of the cerebellar cortex. In this review, we summarize findings identifying TRPC3 channels as integral components of glutamatergic metabotropic synaptic transmission. We give an overview of postsynaptic interaction partners and activation mechanisms of TRPC3 in central neurons. Finally, we address the deleterious consequences of distorted TRPC3 synaptic signaling for cerebellar function in different mouse models and present TRPC3 as an emerging candidate protein implicated in various forms of ataxia in humans.

**Keywords** Cerebellum · Purkinje cell · mGluR1 · STIM1 · Ataxia

The *transient receptor potential* channels derive their name from a *Drosophila* mutant that is characterized by visual impairment. In this *trp* mutant, the photoreceptor light-evoked responses decline to baseline during ongoing illumination. This is in contrast to wildtype photoreceptors that exhibit a steady-state response under the same conditions [1]. The

cloning of the *trp* gene, the analysis of the amino acid sequence, and ensuing electrophysiological studies led to the identification of the TRP protein as a Ca<sup>2+</sup>-permeable light-activated ion channel in the plasma membrane of *Drosophila* photoreceptors [1]. A few years after its discovery in flies, the first mammalian homolog of the *Drosophila* TRP was identified in humans [1]. Work of many laboratories led to the identification of 28 channel subunit genes that were grouped into six protein families [2]. The *classic* TRPs (TRPCs) are the most closely related ones to the original *Drosophila* TRP. The seven members of this protein subfamily (TRPC1–TRPC7) are activated downstream of plasma membrane receptors coupled to G proteins from the G<sub>q</sub>-type and, hence, to signaling cascades that involve phospholipase C (PLC). Like other TRPs, TRPC subunits share similarities with voltage-gated potassium channels. Thus, they possess six transmembrane domains (TM1–6) between their cytosolic N- and C-termini. A pore-forming region is located between TM5 and TM6. By associating as homo- or heterotetramers, TRPC proteins form unspecific cation channels [3].

## General properties of TRPC3 channels in the mammalian brain

TRPC3 is abundantly expressed in the mammalian brain [4–6]. The three different splice variants of *Trpc3* transcripts that were identified so far consist of TRPC3a with 12 exons [7], TRPC3b that lacks the N-terminal exon (exon 0) of TRPC3a [7], and TRPC3c with neither exon 0 nor exon 9 [8]. While neither TRPC3 homotetramers nor TRPC3-containing heteromers were unambiguously found in native cells, there is indirect evidence from co-immunoprecipitation experiments and FRET microscopy that TRPC3 can form homotetramers as well as a preference for the association with

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its closest relatives, TRPC6 and TRPC7 [9]. In addition to that, in different cell types, TRPC3 has been shown to form channels together with TRPC1 and/or TRPC4 [10]. When heterologously expressed in non-excitable cells, TRPC3 is a nonselective cation channel with a moderate preference for  $\text{Ca}^{2+}$  over monovalent cations ( $P_{\text{Ca}}/P_{\text{Na}}=1.6$ ) [11]. The selectivity filter is located in the putative pore loop with a single glutamate residue (E630) determining  $\text{Ca}^{2+}$  permeability [10]. High-resolution cryo-electron microscopy as well as site-directed mutagenesis suggest that the TRPC3 channel gate is located at the level of the inner leaflet of the plasma membrane bilayer [10]. When expressed in non-neuronal cell lines, TRPC3 subunits form channels with a single channel conductance of  $\sim 60$  pA, conducting currents that show both inward and outward rectification and a reversal potential of 0 mV [12]. Similarly to TRPC6 and TRPC7, TRPC3 can directly be activated by diacylglycerol, a product of PLC [13].

## TRPC3 in the cerebellum

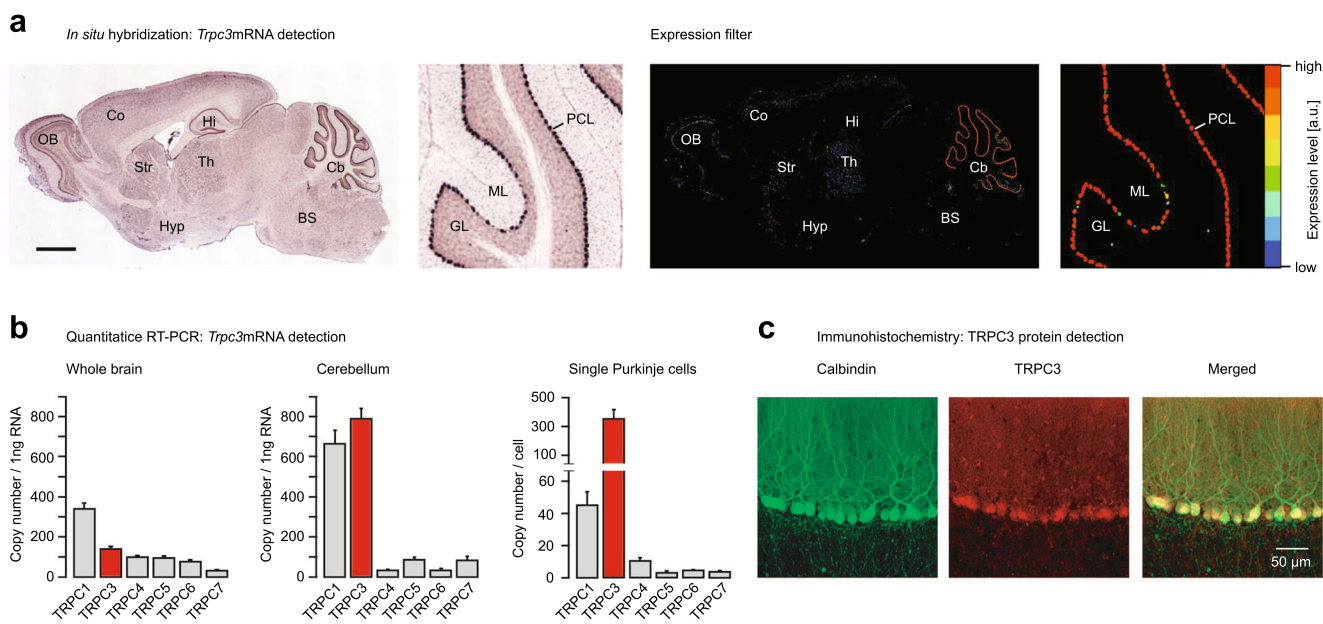
### Expression pattern of TRPC3

In the brain, the expression of TRPC3 is highest in the cerebellum. In mice, rats, and guinea pig cerebella, TRPC3c is the predominant TRPC3 splice variant, followed by TRPC3b. Compared to TRPC3b in heterologous expression systems,

TRPC3c exhibits increased basal and receptor-activated channel currents. It is unclear whether TRPC3a is also expressed in the cerebellum [8]. In situ hybridization studies showed that *Trpc3* mRNA is largely restricted to the Purkinje cell layer (Fig. 1a; [5]). Using a single-cell quantitative real-time (RT)-PCR approach, it was found that in Purkinje cells of adult mice, the expression of *Trpc3* by far outweighs that of other TRPC subunits (Fig. 1b; [4]). Immunohistochemistry analyses indicated that TRPC3 proteins are most abundant in the somatodendritic compartment of Purkinje cells (Fig. 1c; [4]). During postnatal development, there is a significant upregulation of TRPC3 expression in the cerebellum, particularly during the period lasting from postnatal day 1 (P1) to P16 [14]. In the cerebellum, TRPC3 has been detected also in type II unipolar brush cells [15], a class of small excitatory interneurons in the granular layer of the cerebellar cortex [16].

### Consequences of altered *Trpc3* expression in mouse models

The cerebellum plays an important role in the fine-tuning, coordination, and accurate timing of movements as well as in motor learning [17]. In mice with an altered expression of TRPC3 characteristic, clear motoric deficits were observed. Thus, TRPC3-deficient knockout mice show an unusual wide-legged waddling gait. Especially their hind paws are affected by this deficit in motor coordination, which



**Fig. 1** Expression of *Trpc3* in the mouse brain. **a** In situ hybridization demonstrating the presence of *Trpc3* mRNA in a sagittal slice from a mouse brain. *Left*: transmitted light images (*OB* olfactory bulb, *Co* cerebral cortex, *Hi* hippocampus, *Str* striatum, *Th* thalamus, *Hyp* hypothalamus, *BS* brain stem, *GL* granular layer, *ML* molecular layer, *PCL* Purkinje cell layer). *Scale bar*=1500  $\mu\text{m}$ . *Right*: false color representation of different quantitative expression levels (arbitrary units). Images from the Allen Mouse Brain Atlas (<http://mouse.brain->

[map.org/experiment/show/74821603](http://mouse.brain-map.org/experiment/show/74821603); 12th plane). **b** Copy numbers of TRPC subunit mRNA detected in 1 ng total RNA of mouse whole brain (*left*), cerebellum (*middle*), and in single Purkinje cells (*right*; mean $\pm$ SEM). **c** A dual-channel confocal scan of an immunohistochemical staining in an acute cerebellar slice. Calbindin-D28k immunoreactivity is shown in *green* (*left*) and that for TRPC3 is shown in *red* (*middle*). *Right*: merged images. (*b*) and (*c*) are modified from [4]

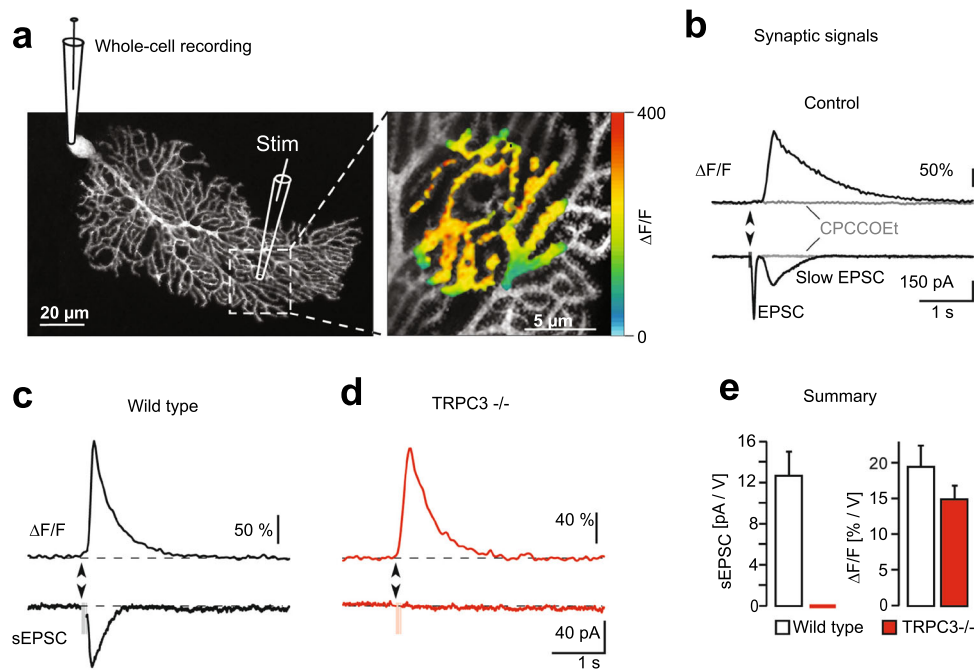
significantly increases the number of hindpaw slips when mice walk on a horizontal ladder or a thin horizontal beam, respectively [4]. We conclude that TRPC3 is important for cerebellar function. Electrophysiological analyses of Purkinje cells demonstrated a complete lack of the characteristic slow excitatory postsynaptic current (slow EPSC; [4]) known to involve activation of subtype I metabotropic glutamate receptors (mGluR1; [18]) (Fig. 2b). In contrast, “fast” excitatory synaptic transmission, mediated by AMPA receptors, was found to be unaltered in the absence of TRPC3 [4]. These results established for the first time that a TRP superfamily member is indispensable for normal brain function.

The importance of TRPC3 for mGluR1-dependent synaptic transmission and sensorimotor processing for normal behavior was further substantiated by the analysis of *Moonwalker* (*Mwk*) mice. These mice harbor a spontaneous point mutation (T635A) in the same exon that was excised in the TRPC3-deficient knockout mice (exon 7; [4]). The *Mwk* mutation results in a single amino acid exchange and the loss of a phosphorylation site in the TRPC3 protein. As a result, the gating of the channel is altered and currents through TRPC3 are increased. Homozygous *Mwk/Mwk* mice are not viable. Heterozygous *Mwk/+* mice suffer from a serious form of ataxia. Remarkably, the behavioral defects are much more pronounced in these mice with an increased function of TRPC3 than in those that lack TRPC3 [19]. While

development and morphology of Purkinje cells is largely normal in the absence of TRPC3 [4], in *Mwk/+* mice, the outgrowth of the dendritic tree of these cells is strongly reduced. Furthermore, there is a slow but progressive decline of Purkinje cell number in *Mwk/+* mice starting at 4 months of age [19]. The ataxic phenotype, in contrast, is present already before weaning. Interestingly, there is almost a complete loss of TRPC3-expressing type II unipolar brush cells in *Mwk/+* mice at 1 month of age [15]. Moreover, already 3 weeks after birth, most Purkinje cells in these mice were strongly depolarized ( $V_m = -33$  mV) in acute cerebellar slices, and, possibly due to a depolarization block, many of them stopped firing action potentials [15]. Thus, the loss of unipolar brush cells together with Purkinje cell electrophysiological dysfunction may underlie the early onset of the *Mwk* phenotype [15].

### Activation mechanism of TRPC3

Postsynaptic mGluR1 are particularly abundant at parallel fiber-Purkinje cell synapses [20]. The requirement of intact mGluR1-mediated synaptic signaling for sensorimotor processing in the cerebellum is supported by various studies [21]. The TRPC3-mediated slow EPSC is one of two types of synaptic signals that are activated downstream of activation of mGluR1 during high-frequency firing of parallel fibers [21] (Fig. 2a). The molecular link between mGluR1 and TRPC3 is



**Fig. 2** Synaptic mGluR1-mediated Purkinje cell signaling in the absence of TRPC3. **a** Right: confocal image of a patch-clamped Purkinje cell in a cerebellar slice from a wildtype mouse. Left: the site of electrical stimulation (Stim) at higher magnification. Pseudocolor image of a synaptic  $Ca^{2+}$  signal evoked by parallel fiber stimulation. **b** Black traces: PF-evoked (five pulses, 200 Hz, in 10  $\mu M$  CNQX) synaptic response consisting of an early rapid and a slow EPSC (bottom) and a  $Ca^{2+}$

transient (top). Grey traces: block of slow components by the mGluR-antagonist CPCCOEt (200  $\mu M$ ). **c** Slow EPSC in a wildtype mouse (lower trace) and the corresponding local dendritic  $Ca^{2+}$  response (upper trace). **d** Similar recording in a TRPC3-deficient knockout mouse. **e** Summary graphs for normalized (to stimulation strength) sEPSC and  $Ca^{2+}$  transients ( $\Delta F/F$ ). Modified from [4]

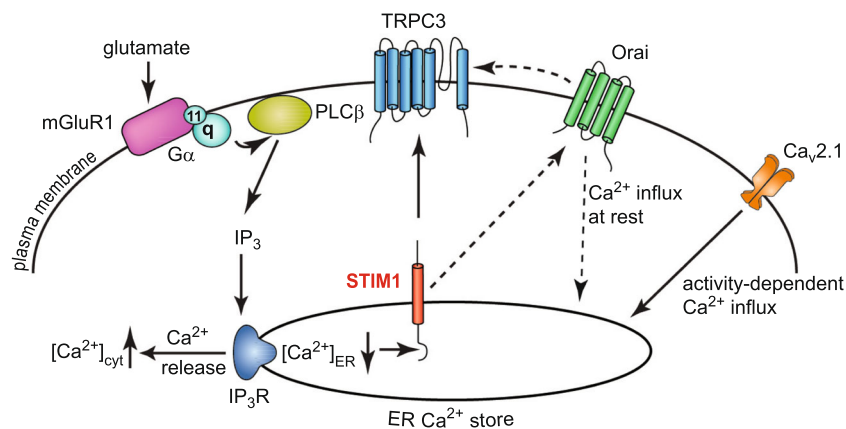
only partially understood. Much better clarified is the mechanism underlying mGluR1-dependent  $\text{Ca}^{2+}$  release from endoplasmic reticulum (ER)  $\text{Ca}^{2+}$  stores in spines and dendrites [22–24]. The two signal components of mGluR1-dependent synaptic transmission, consisting of a slow EPSP and  $\text{Ca}^{2+}$  release signal from internal stores, are thought to involve distinct intracellular signaling cascades [4, 23–26]. However, both mGluR1-dependent signal components are mediated by trimeric G proteins of the  $G_q$  family. While the intracellular  $\text{Ca}^{2+}$  release signal relies primarily on  $G\alpha_q$ , both  $G\alpha_q$  and  $G\alpha_{11}$  are involved in the generation of the TRPC3-mediated slow EPSC [27]. The  $G_q$ -dependent activation of the phospholipase  $C\beta$  (PLC $\beta$ ) mediates the accumulation of inositol trisphosphate ( $\text{IP}_3$ ) in the cytosol and, thus, the opening of  $\text{IP}_3$  receptor channels in the ER, through which  $\text{Ca}^{2+}$  ions are released into the cytosol [22, 23]. Whether the two signaling cascades leading to postsynaptic  $\text{Ca}^{2+}$  release or TRPC3 activation, respectively, diverge before or after the PLC $\beta$ , is unclear. While the slow TRPC3-dependent EPSC was found to be resistant to blockade of the PLC $\beta$  by its antagonist U73122 [26, 28, 29], it was absent in PLC $\beta$ 4-deficient knockout mice in those cerebellar lobules in which PLC $\beta$ 4 is the predominant PLC $\beta$  isozyme [30]. The PLC $\beta$ -dependent pathway may exist, therefore, independently from one that involves another PLC version, the PLD1 [28]. Indeed, TRPC3-mediated slow EPSCs depend on the activation of the PLD1 by Rho-GTPases downstream of mGluR1. Another study indicated that both PLC and PLD can activate heterologously expressed TRPC3 channels through DAG [31]. However, the DAGs produced by both phospholipases differ in their chemical structure. In contrast to PLD-derived DAG, DAG that results from PLC activity has the potency to activate the protein kinase C (PKC) that negatively regulates TRPC3 in expression systems [32]. Native TRPC3 channels expressed in Purkinje cells, however, are not affected by the activity of PKC [33]. Together, these observations provide support for a role of PLD in TRPC3 activation.

Our group recently identified the *stromal interaction molecule 1* (STIM1) as a key regulator of slow mGluR1-

dependent synaptic transmission mediated by TRPC3 [34]. STIM1 was originally found in a screen for stromal cell gene products that bound to precursors of B lymphocytes. The sequence of the *Stim1* gene is conserved from *Drosophila* to mammalian species and appears to be expressed ubiquitously in human tissues. In non-excitable cells, STIM1 plays a central role in intracellular  $\text{Ca}^{2+}$  homeostasis. In terms of its molecular structure, STIM1 has a single transmembrane domain and is localized primarily to the ER membrane where it functions as a  $\text{Ca}^{2+}$  sensor that determines the filling state of ER  $\text{Ca}^{2+}$  stores. When the  $\text{Ca}^{2+}$  concentration in the stores decreases, STIM1 activates Orai channels in the plasma membrane, which, by acting as  $\text{Ca}^{2+}$  release activated  $\text{Ca}^{2+}$  (CRAC) channels, mediate the  $\text{Ca}^{2+}$  influx required for store refilling (Fig. 3). In addition to that, STIM1 has been shown to interact with TRPC channels [35].

Cerebellar Purkinje cells abundantly express STIM1 [5, 34–36]. Mice with a Purkinje cell-specific deletion of *Stim1* (STIM1<sup>pk0</sup> mice) have pronounced alterations in cerebellar motor control. Thus, during repeated runs on an accelerated rotarod, they show no performance improvement, as typically observed in wildtype mice, and they slip more often with their hind paws while traversing a horizontal beam [34]. Electrophysiological and  $\text{Ca}^{2+}$  recordings from Purkinje cells of STIM1<sup>pk0</sup> mice showed that both types of mGluR1-dependent synaptic signals, the  $\text{IP}_3$  receptor-dependent  $\text{Ca}^{2+}$  release from stores and the TRPC3-mediated slow EPSC, are virtually abolished. At resting membrane potential ER  $\text{Ca}^{2+}$  stores were found to be depleted [34].  $\text{Ca}^{2+}$  influx through voltage-gated  $\text{Ca}^{2+}$  channels (VGCCs), however, can render STIM1-deficient Purkinje cells responsive to an mGluR1-specific agonist and can restore both  $\text{Ca}^{2+}$  release and the inward current through TRPC3. Thus, STIM1 at resting membrane potential has a regulatory role in store refilling through Orai channels that are present in Purkinje cells [34] but does not prevent store filling through VGCCs [37]. These results demonstrate that, rather than through a direct intermolecular electrostatic interaction as it was shown in non-neuronal cells [38], in cerebellar Purkinje cells, STIM1 controls TRPC3

**Fig. 3** Schematic representation of the actions of STIM1 in cerebellar Purkinje cells. From ref. [34]



opening downstream of mGluR1 through the regulation of intracellular  $\text{Ca}^{2+}$  that might have a permissive role for the activation of native TRPC3 in neurons [12].

TRPC3 channels that are expressed heterologously in non-neuronal cell channels can open upon their binding to the N-terminal domain of the activated  $\text{IP}_3$  receptor [39]. In Purkinje cells, such a mechanism of TRPC3 gating is unlikely, because in  $\text{IP}_3$  uncaging experiments [34], activation of  $\text{IP}_3$  receptors failed to produce an inward current. The discrepancy between both findings may be explained by the predominant expression of TRPC3c in Purkinje cells that has only a truncated *calmodulin and IP<sub>3</sub> receptor binding* (CIRB) domain [8].

### Role of TRPC3 for cerebellar function

*Trpc3* expression in Purkinje cells is strongly upregulated during postnatal development associated with intensive dendritic outgrowth [14]. Most remarkably, absence of TRPC3 leaves dendrites unaltered in size and general morphology [4]. Instead, excessive activation of mGluR1 inhibits dendritic growth of Purkinje cells in cerebellar slice cultures. This effect, however, is not mediated by TRPC3 [40]. These observations suggest that TRPC3 is not required for normal dendritic development in Purkinje cells. Increased TRPC3 activity, however, can strongly impair dendritic development in *Mwk*<sup>+/+</sup> mice [19].

Motor learning is thought to involve persistent synaptic changes in the cerebellum. The best studied type of synaptic plasticity is cerebellar long-term depression (LTD), the long-lasting reduction of transmission efficiency at parallel fiber to Purkinje cell synapses. LTD is induced by the conjunctive activity of parallel and climbing fibers. Signaling by mGluR1 is critically required for the postsynaptic processes underlying LTD [41]. Downstream effectors of mGluR1 include TRPC3 [42]. In rat cerebellar slices, it was shown that LTD is largely reduced when TRPC3 is blocked pharmacologically or by a specific antibody. The blockade of TRPC3 inhibits processes that underlie LTD induction, namely postsynaptic  $\text{Ca}^{2+}$  changes, the activation of PKC, and the internalization of the AMPA receptor subunit GluR2 [42].

A role of TRPC3 in cerebellar output firing is suggested by recordings in vivo. In awake mice, Purkinje cells lacking the biomarker zebrin II that defines parasagittally organized cerebellar zones [43] were found to fire at a significantly higher frequency (90 Hz) than zebrin-positive Purkinje cells (60 Hz). Although TRPC3 itself does not share zebrin-like expression patterns [4, 15, 19], this firing frequency difference was attenuated by the pharmacological blockade of TRPC3 [44]. Thus, normal TRPC3-signaling contributes to the spike firing of Purkinje cells in vivo.

In conclusion, the available experimental evidence clearly identifies TRPC3 as a key molecule in the mammalian brain that regulates Purkinje cell signaling and cerebellar function.

During recent years, our knowledge about TRPC3 in Purkinje cells has greatly increased, but major open questions remain. These include, for example, the synaptic gating mechanism of TRPC3, the contribution of TRPC3 to postsynaptic  $\text{Ca}^{2+}$  signaling, its actions in the olivocerebellar circuit in vivo, and whether it has distinct properties at parallel and climbing fiber synapses of Purkinje cells, respectively.

### Relevance of TRPC3 for diseases in humans

In postmortem human cerebella from individuals aged from 8 days to 83 years, TRPC3 protein was detected throughout the entire lifespan, without age-dependent changes in expression levels [45]. A first indication that *Trpc3* is a candidate gene for cerebellar pathologies in humans came from the *staggerer* (*sg*) mouse line, which is an extreme mouse model for spinocerebellar ataxia 1 (SCA1). The progressive development of SCA1 symptoms (gait abnormalities, incoordination, and problems with speech, swallowing, and breathing) is strongly correlated with a dysfunction in the transcription factor *RoR $\alpha$*  that is abundantly expressed in Purkinje cells [46]. The *sg* mouse carries a spontaneous mutation in *ROR $\alpha$* . These mice lack mGluR1-mediated slow EPSCs completely. Neither intense parallel fiber stimulation nor an exogenously applied mGluR agonist can elicit mGluR1-mediated responses in cerebellar slices from *sg/sg* mice. TRPC3 and mGluR1 show diminished expression, and mGluR1 is mislocalized [47]. This suggests that disruption of TRPC3-signaling may occur in SCA1 pathology. However, an initial genetic screen for *Trpc3* mutations in 98 patients with genetically undefined late-onset cerebellar ataxia and ten patients with undefined episodic ataxia did not reveal causative mutations in *Trpc3* [48].

Consistent with the cell type-specific and developmental regulation of the gene, an alternative promotor for *Trpc3* was identified encompassed by a so-called CpG island, a site where allele-specific methylation and, thus, silencing of the gene occurs. The methylation status of the *Trpc3* alternative promotor is determined by a single nucleotide polymorphism (SNP) in this region. In a genetic screen, the genotype with the homozygous rare unmethylated allele was found at significantly higher frequencies in patients with idiopathic ataxias [49]. Another study reported a point mutation in the *Trpc3* gene (R762H) in a patient with late-onset unidentified ataxia. This site is located in the so-called TRP-domain of the TRPC3 protein that is important for channel gating [10]. When the mutant gene was expressed in mice, this induced neuronal cell death [50] suggesting a deleterious gain-of-function mutation similarly to the *Mwk* mutation. In conclusion, various lines of evidence indicate that mutations in *Trpc3* may play an important role for a class of ataxia found in humans. Studies performed in animal models provide strong support that a key

pathophysiological mechanism for the disease is the impaired mGluR-dependent TRPC3-mediated slow EPSP in cerebellar Purkinje cells.

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