



# Calretinin: from a “simple” Ca<sup>2+</sup> buffer to a multifunctional protein implicated in many biological processes

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The hexa-EF-hand Ca<sup>2+</sup>-binding protein calretinin (CR) is predominantly expressed in specific neurons of the central and peripheral nervous system. However, CR expression is also observed in non-neuronal cells, e.g., during embryonic development and in mesothelioma cells. Of the 6 EF-hand domains, 5 are functional; the first 4 domains form 2 pairs showing high cooperativity within a pair that results in non-linear modulation of intracellular Ca<sup>2+</sup> signals by CR. EF-hand domain 5 has a low affinity and represents the identified interaction site with CR-binding partners present in mouse cerebellar granule cells. CR binding to other targets including the pore-forming  $\alpha_1$  subunit of the Ca<sup>2+</sup> channel Ca<sub>v</sub>2.1, as well as to huntingtin indicates additional Ca<sup>2+</sup> sensor functions besides the well-known Ca<sup>2+</sup>-buffering functions. The absence of CR in cerebellar granule cells of CR<sup>-/-</sup> mice results in increased excitability and altered firing of Purkinje cells and promotes cerebellar 160-Hz oscillations impairing motor coordination. The putative role of CR in neuroprotection is still highly discussed. Altogether, CR emerges as a multi-functional protein also associated with development, i.e., cell proliferation, differentiation, and cell death.

**Keywords:** calretinin, calcium signaling, calcium sensor, calcium buffer, neuron excitability

## BASIC FACTS ABOUT CALRETININ (CR)

Calretinin (CR; human gene symbol: *CALB2*), calbindin D-28k (CB; *CALB1*) and secretagogin (SCGN; *SCGN*) represent the 3 members of the hexa-EF-hand protein family, also named the calbindin sub-family [see recent reviews on CR (Camp et al., 2009), CB (Schmidt, 2012) and SCGN (Alpar et al., 2012)]. They all contain 6 structural motifs named EF-hand Ca<sup>2+</sup>-binding domains. Each domain consists of an alpha-helix of approximately 10 amino acids, a Ca<sup>2+</sup>-chelating loop of 12 amino acids and a second alpha-helix that is oriented perpendicular to the first one (for more details on the EF-hand structure, see (Schwaller, 2010). CR (M<sub>r</sub> 31 kDa) initially discovered in the retina, thus the name: calcium + retina = CR consists of 271 amino acids in many species and is highly conserved; the number of amino acids varies from 269 (e.g., *Gallus gallus*; Chicken) to 273 (e.g., *Monodelphis domestica*; Gray short-tailed opossum). CR is also expressed in zebrafish (*Danio rerio*) and an invertebrate ortholog named calbindin 53E (*Cbp53E*; previously *calbindin-32*) exists in *Drosophila melanogaster* that shares the highest sequence identity with CR (Reifegerste et al., 1993). In CR, the first 5 EF-hand domains are capable of binding Ca<sup>2+</sup> ions, while the sixth one is inactive (Stevens and Rogers, 1997; Schwaller et al., 1997). Moreover the Ca<sup>2+</sup>-binding affinity for site 5 is very low (K<sub>D</sub>: 36  $\mu$ M) (Faas et al., 2007) indicating that this site is expected to be rarely in the Ca<sup>2+</sup>-bound form in the cytoplasmic compartment except in microdomains close to Ca<sup>2+</sup> channels. The other 4 functional Ca<sup>2+</sup>-binding sites form 2 similar pairs likely consisting of domains 1 and 2, as well as 3 and 4 showing strong cooperativity within a pair (Faas et al., 2007). The apparent K<sub>D</sub>

(K<sub>D, app</sub>) for the 4 sites is 1.4–1.5  $\mu$ M with high cooperativity (n<sub>H</sub> of 1.9; for more details on CR's properties, see **Table 1**). This property results in non-linear Ca<sup>2+</sup> regulation in cells due to the presence of CR. In a situation when the intracellular Ca<sup>2+</sup> concentration [Ca<sup>2+</sup>]<sub>i</sub> is at resting (basal) levels of 50–100 nM, then upon a brief and limited increase in [Ca<sup>2+</sup>]<sub>i</sub>, CR behaves like a typical slow-onset buffer (EGTA). However, if the same increase occurs at elevated [Ca<sup>2+</sup>]<sub>i</sub>, in the order of 1  $\mu$ M, when the first site of a pair is in the Ca<sup>2+</sup>-bound form, cooperativity sets in and CR functions almost like the fast buffer BAPTA (for more details on this behavior, e.g., on the spatiotemporal patterns of IP<sub>3</sub>-evoked Ca<sup>2+</sup> signals, see Dargan et al. (2004) or on CR's role modeled for a train of intracellular Ca<sup>2+</sup> signals, see Figure 3 in (Schwaller, 2009). Thus, the Ca<sup>2+</sup>-binding kinetics of CR strongly depends on [Ca<sup>2+</sup>]<sub>i</sub> levels at the time when another increase in [Ca<sup>2+</sup>]<sub>i</sub> occurs. Besides these novel properties of Ca<sup>2+</sup> binding in a protein, first described for CR, several studies in the 90's reported CR to undergo considerable Ca<sup>2+</sup>-dependent conformational changes, which indicated that CR might also have “Ca<sup>2+</sup> sensor” functions like the prototypical sensor calmodulin (CaM). Results in support of CR acting as a Ca<sup>2+</sup> sensor are presented in Section III.

Up to date, no structural data of full-length CR have been reported. However, the NMR structure of the N-terminal 100 amino acids of rat CR (Palczewska et al., 2001) embracing EF-hand domains 1 and 2 are very similar to the NMR solution structure of the corresponding domains in rat CB (Kojetin et al., 2006). Together with the similar results from limited proteolysis experiments obtained with CR and CB, this suggests that

**Table 1 | Properties of calretinin (modified from Schwaller, 2009, 2010, 2012).**

General parameters	Value (range)	References/comments
Amino acids	269–273; 271 in most mammals; 310 <sup>a</sup>	Reifegerste et al., 1993; Zimmermann and Schwaller, 2002
Molecular mass ( $M_r$ )	30–31 kDa	Rogers, 1987
EF-hand domains	6	Rogers, 1987
Functional $Ca^{2+}$ -specific sites	5	Stevens and Rogers, 1997; Schwaller et al., 1997
Identified CR binding partners	$\alpha_1$ subunit of $Ca_v2.1$ , huntingtin	Christel et al., 2012; Dong et al., 2012
<b>METAL BINDING PROPERTIES AND MOBILITY</b>		
$K_{D, Ca}$	$K_{D(T)}$ 28 $\mu M$ $K_{D(R)}$ 68 nM $K_{D(app)}$ 1.4 $\mu M$ EF5: 36 $\mu M^b$	CR has 2 cooperative pairs (sites EF1-4) with indistinguishable binding properties. In the absence of $Ca^{2+}$ , the first site within a pair is in the tensed (T) state and changes upon $Ca^{2+}$ binding of the first site within a pair to the relaxed (R) state. These results were obtained by flash photolysis experiments Faas et al., 2007
$K_{D, Mg}$	4.5 mM	Stevens and Rogers, 1997
$k_{on, Ca}$ ( $\mu M^{-1} s^{-1}$ )	Tensed (T) sites: 1.8 Relaxed (R) sites: 310 EF-hand domain 5: 73	Faas et al., 2007
Cooperativity	Yes $n_H \approx 1.3$ –1.9	Stevens and Rogers, 1997; Faas et al., 2007
Mobility $D_{C_{buffer}}$ ( $\mu m^2 s^{-1}$ ) in $H_2O$	$120 \pm 1$ (means $\pm$ s.e.m.)	Arendt et al., 2013
$D_{10ms}$ ( $\mu m^2 s^{-1}$ ) <sup>c</sup>	3.2 (IQR 1.6–5.9)	
<b>INTRACELLULAR CONCENTRATION IN</b>		
Frog saccular hair cells	1.2 mM	Edmonds et al., 2000
Rat outer hair cells	35 $\mu M$	Hackney et al., 2005
Rat inner hair cells	20 $\mu M$	Hackney et al., 2005
Mouse cerebellar granule cells	30–40 $\mu M^d$ ; 0.7–1.2 mM <sup>e</sup>	Gall et al., 2003

<sup>a</sup>*Drosophila melanogaster calbindin 53E (previously calbindin-32) shows the highest sequence homology to calretinins of different species.*

<sup>b</sup>*A lower affinity for EF5 ( $K_D$ : 0.5 mM) was determined with the flow dialysis method (Schwaller et al., 1997).*

<sup>c</sup>*Diffusion determined in cerebellar granule cell dendrites by an anomalous subdiffusion model.*

<sup>d</sup>*Based on BAPTA concentration (150  $\mu M$ ) needed to restore granule cell excitability in  $CR^{-/-}$  cells.*

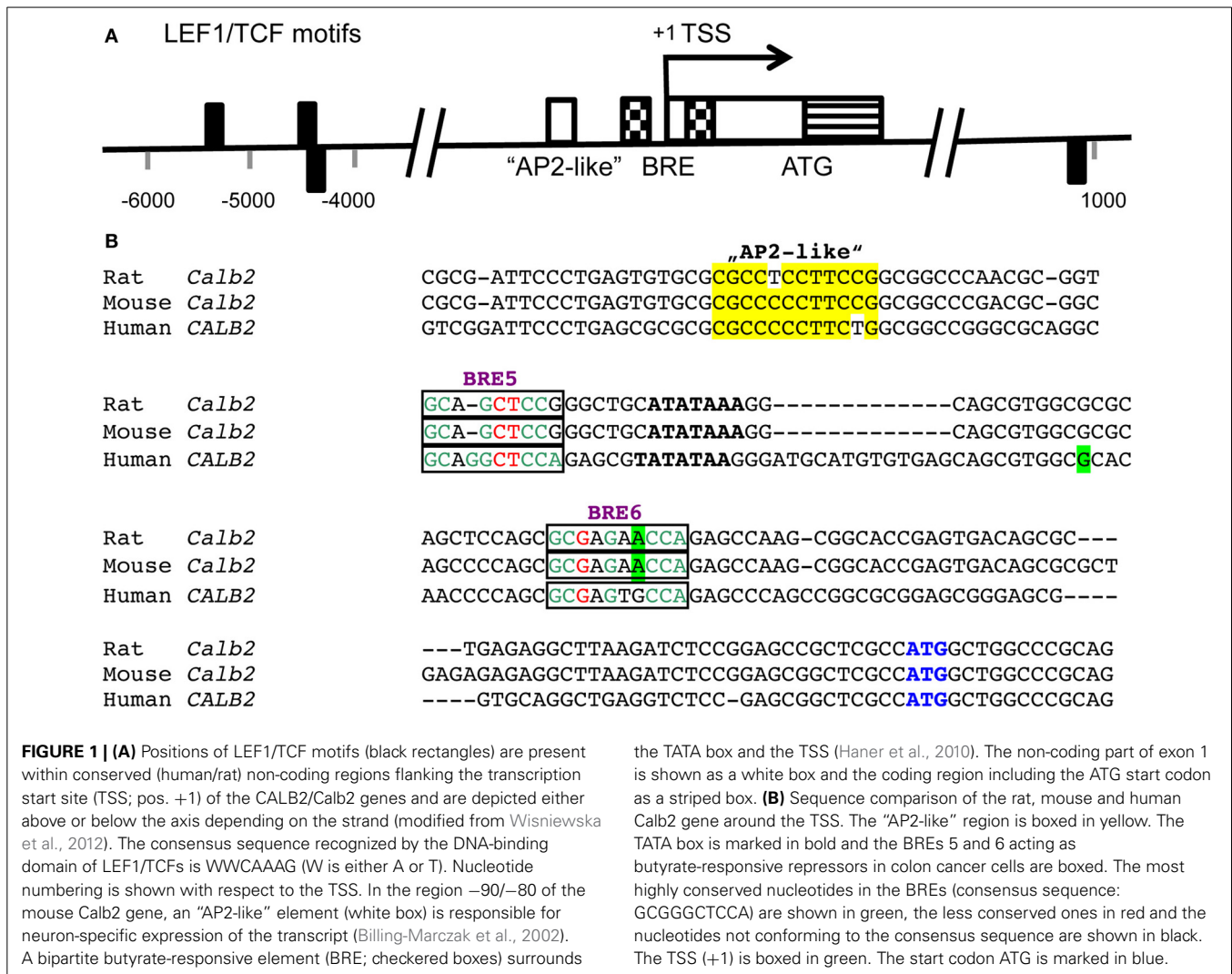
<sup>e</sup>*Based on numerical simulations of buffered  $Ca^{2+}$  diffusion near a single  $Ca^{2+}$  channel or a large cluster of  $Ca^{2+}$  channels (Safteku, 2012).*

the overall structure of hexa-EF-hand proteins might be rather similar.

## REGULATION OF CALRETININ EXPRESSION

Still relatively little is known on the mechanisms of regulation of CR expression in various tissues; altered CR expression levels have been reported as the consequence of experimental manipulations or are associated with certain diseases in humans and/or animal models of these diseases [for more details, see Schwaller, 2009, 2010, 2012]. Based on the substantial sequence homology in the promoter region including the TATA and CAAT boxes of the human *CALB2* and mouse *Calb2* gene (Strauss et al., 1997), it is reasonable to assume that CR expression is regulated in a similar manner in the two species, although species differences in CR expression have been reported before. Neuron-specific “CR-like” expression of a luciferase reporter gene in cortical cultures is achieved in the presence of the mouse *Calb2* promoter region from  $-115/+54$ . The 5' region of this promoter fragment ( $-115/-71$ ) selectively binds a nuclear protein present in cerebellar granule cells and contains an “AP2-like” element ( $-90/-80$  bp; **Figure 1**). This element is essential for the neuron-specific reporter expression (Billing-Marczak et al., 2002). The same “AP2-like” element doesn't affect transcriptional activity in human colon carcinoma and mesothelioma cells indicating

that CR expression in neurons and non-neuronal cell types is differently regulated (Billing-Marczak et al., 2004). In human colon cancer cells, CR expression is downregulated by butyrate (Marilley et al., 2001), a substance derived from intestinal fermentation of dietary fibers by bacteria. Butyrate, a known modulator of histone acetylation, inhibits the cell cycle and leads to enterocyte differentiation. Of the several putative butyrate-responsive elements (BREs) present in the human *CALB2* promoter, two elements embracing the TATA box act as butyrate-sensitive repressor elements in colon cancer cells, but not in cells of mesothelial origin (**Figure 1**; Haner et al., 2010). This supports the notion of cell type-specific *CALB2* regulation. The rat *Calb2* promoter region contains 3 binding motifs for the transcription factor LEF1/TCF that binds to  $\beta$ -catenin via its N-terminal region (**Figure 1**);  $\beta$ -catenin, not directly binding to DNA, contains a strong trans-activation domain and is highly expressed in thalamic neurons. Down-regulation of  $\beta$ -catenin by its negative regulator Axin2 significantly reduces CR expression in cultured rat thalamic neurons indicating that  $\beta$ -catenin is a positive regulator of the *Calb2* gene (Wisniewska et al., 2012). In addition, several transcripts exist from the human *CALB2* gene (Schwaller et al., 1995), which are present in several colon cancer cell lines (Gander et al., 1996) and in tumor tissue from primary colon tumors (Schwaller et al., 1998). One splice variant with deletion of exons 8 and 9 results



in a truncated protein named CR-22k of 192 amino acids, which is expressed in certain tumors (Schwaller et al., 1998); the other transcript (deletion of exon 8) is currently known to exist only at the level of mRNA (Schwaller et al., 1995) and might have a function as an RNA molecule, possibly as a target for micro (mi)RNA or acting as a long non-coding (lnc) RNA.

### THE IDENTIFICATION OF CALRETININ-INTERACTING TARGETS SUPPORTS CR'S ROLE AS A Ca<sup>2+</sup> SENSOR

The finding that purified CR undergoes significant Ca<sup>2+</sup>-dependent conformational changes *in vitro* (Kuznicki et al., 1995; Schwaller et al., 1997), together with the observation that CR immunoreactivity in chick brainstem auditory neurons changes from diffuse cytosolic staining to intense localized staining beneath the plasma membrane, which occurs together with the onset of spontaneous activity (Hack et al., 2000), suggested already in the late 90's that CR might have additional Ca<sup>2+</sup>-sensor functions (Billing-Marczak and Kuznicki, 1995). Furthermore, CR was shown to be present in membrane fractions of rat cerebellum (Winsky and Kuznicki, 1995) and to bind to

cytoskeletal elements in WiDr colon cancer cells (Marilley and Schwaller, 2000). In support of the presence of CR targets, CR translocates from the cytosol to the nucleus in a vitamin D<sub>3</sub>-dependent and/or butyrate-dependent way in colon cancer cells *in vitro*, also indicating that CR might have nuclear interaction partners (Schwaller and Herrmann, 1997). Recently, in two studies CR targets were identified (Christel et al., 2012; Dong et al., 2012) and moreover, the interacting domain of CR that leads to a decreased Ca<sup>2+</sup>-dependent mobility of CR in cerebellar granule cells was identified (Arendt et al., 2013). A yeast-two-hybrid screen with CR as bait, identified a consensus, strongly basic peptide sequence H(R/K)HRRR(E/D) as a putative CR-binding (CRB) motif (Christel et al., 2012). This motif is present in multiple copies (CRB1-5) in the cytoplasmic linker region between domains II and III of the channel-forming alpha 1 subunit of the high-voltage activated Ca<sup>2+</sup> channel Cav2.1 (P/Q type). This channel is regulated in a dual fashion by Ca<sup>2+</sup> ions, showing both, Ca<sup>2+</sup>-dependent inactivation (CDI) and Ca<sup>2+</sup>-dependent facilitation (CDF); both mechanisms influence synaptic plasticity in the nervous system. In cells expressing Cav2.1 *in vitro*,

co-expression of CR inhibits CDI and enhances CDF via a direct interaction with the  $\alpha_12.1$  subunit. The channel subunit  $\alpha_12.1$  co-immunoprecipitates with CR antibodies using either extracts from HEK293T cells transfected with CR and  $\text{Cav}2.1$  or mouse cerebellar extracts. In mouse cerebellum, both CR and  $\text{Cav}2.1$  are strongly expressed in granule cells and the absence of CR in  $\text{CR}^{-/-}$  mice causes impairment in motor control (Schiffmann et al., 1999). This impairment is essentially caused by CR's absence in granule cells, since the motor phenotype can be rescued by selective re-expression of CR in granule cells (Bearzatto et al., 2006). Thus, the direct modulation of  $\text{Cav}2.1$  by CR affects intracellular  $\text{Ca}^{2+}$  signaling and probably also neuronal excitability via a mechanism that is different from CR's previously proven  $\text{Ca}^{2+}$  buffering function as discussed in Section IV. The interaction between CR and interacting partners likely including  $\text{Cav}2.1$  was studied in granule cells by fluorescence recovery after photobleaching (FRAP). The diffusion of fluorescence-labeled CR molecules is much slower than the one of freely diffusible molecules (fluorescein dextrans) of comparable size (Arendt et al., 2013). Moreover, during a burst of action potentials (APs) that leads to an increase in dendritic  $[\text{Ca}^{2+}]_i$ , CR's mobility is further decreased, indicative of  $\text{Ca}^{2+}$ -dependent interactions. Addition of a peptide consisting of EF-hand 5 of CR to granule cells, considerably increases CR's mobility implicating that the CR interactions occur mainly via the region of EF-hand 5, the  $\text{Ca}^{2+}$ -binding site with very low affinity. Estimations on the density (concentration) of  $\text{Cav}2.1$  channels and CR in granule cells indicate that channel numbers are too low to account for the strong effect on CR's mobility implicating additional, yet unidentified CR-binding partners in these neurons. A binding partner interacting with CR was found to be huntingtin (Htt), identified by tandem affinity purification (Dong et al., 2012). Binding to CR is even stronger with a mutant form of Htt characterized by a polyglutamine (polyQ) region that is typical for Huntington's disease (HD). In neuronal cultures, CR colocalizes with Htt and a CR/Htt complex can be isolated by co-immunoprecipitation. In CR-overexpressing HEK293 cells, levels of phosphorylated AKT (p-AKT) are increased. At the functional level, CR overexpression reduces mHtt-related  $\text{H}_2\text{O}_2$  cytotoxicity in various HD *in vitro* models. This might be directly linked to CR's capacity to decrease  $[\text{Ca}^{2+}]_i$  in these cells and/or to indirectly increase levels of p-AKT considered as a pro-survival signal. On the other hand, CR down-regulation by shRNA enhances mHtt-mediated neuronal cell death. Based on their findings, the authors conclude that "CR may be a potential therapeutic target for treatment of HD." A link between CR and p-AKT was reported before; increased expression levels of CR strongly correlate with increased resistance to asbestos cytotoxicity in immortalized Met-5A mesothelial cells (Henzi et al., 2009). This protective effect is abrogated in the presence of phosphatidylinositol 3-kinase (PI3K) inhibitors, in support of the above findings that increased PI3K/AKT signaling (increased p-AKT) caused by CR up-regulation favors cell survival. Thus, in the case of CR-expressing mesothelial and mesothelioma cells, CR or more precisely its down-regulation, might be viewed as a potential new target/strategy for malignant mesothelioma therapy (Blum and Schwaller, 2013).

## THE EFFECT OF CALRETININ ON INTRACELLULAR $\text{Ca}^{2+}$ SIGNALING

The particular  $\text{Ca}^{2+}$ -binding properties of CR together with its mobility differently affect intracellular  $\text{Ca}^{2+}$  signals, however only to a measurable extent, if present at a sufficiently high concentration, typically in the range of tens of  $\mu\text{M}$  in neurons. Generally, lower CR concentrations ( $\approx 1 \mu\text{M}$ ) don't affect  $\text{Ca}^{2+}$  signals and e.g., don't protect PC12 cells against  $\text{Ca}^{2+}$  overload induced by ionophore treatment or trophic factor deprivation (Kuznicki et al., 1996). Effects of CR on  $\text{Ca}^{2+}$  signals are often deduced from comparing signals in neurons from WT and  $\text{CR}^{-/-}$  mice (Schmidt et al., 2013) or when overexpressing or down-regulating CR in cell culture models (Billing-Marczak et al., 1999; D'Orlando et al., 2001; Pecze et al., 2013). CR's particular properties, i.e., its low  $\text{Ca}^{2+}$  occupancy at resting  $[\text{Ca}^{2+}]_i$  together with the high cooperativity resulting in non-linear binding properties in a setting in which neurotransmitter release depends supralinearly on  $\text{Ca}^{2+}$  (e.g., in parallel-fiber (PF) terminals onto Purkinje cells) result in considerable nanodomain  $\text{Ca}^{2+}$ -buffering by CR. As a consequence, a minor increase in the amplitude of AP-evoked  $\text{Ca}^{2+}$  signals in  $\text{CR}^{-/-}$  PF boutons results in a considerably higher release probability (Schmidt et al., 2013). CR-deficient cerebellar granule cells are characterized by faster APs and, when electrically stimulated to generate repetitive spike discharges, show enhanced frequency increase with injected currents, i.e., increased excitability (Gall et al., 2003). The excitability can be reverted to the situation seen in WT cells, by loading the cells with the fast buffer BAPTA ( $150 \mu\text{M}$ ) strongly indicating that the "fast"  $\text{Ca}^{2+}$  buffering function of CR is most likely responsible for limiting granule cell excitability. From these experiments it was also deduced that the CR concentration in these neurons is in the order of  $40 \mu\text{M}$ , based on CR's 4 high-affinity  $\text{Ca}^{2+}$ -binding sites; this estimation is in line with modeling studies on CR function (Roussel et al., 2006). However, other models taking into account CR's cooperativity of  $\text{Ca}^{2+}$  binding resulting in a delayed equilibration with  $\text{Ca}^{2+}$  predict the concentration of CR to be even higher, in the order of  $0.7\text{--}1.2 \text{ mM}$  (Saftenku, 2012), a value estimated to be present in frog saccular hair cells (Edmonds et al., 2000). However, in this model the modulation of the main voltage-activated  $\text{Ca}^{2+}$  channel in granule cells,  $\text{Cav}2.1$ , by CR (Christel et al., 2012) was not taken into account. Thus, the precise concentration of CR in granule cells has to be determined yet, possibly by a *in situ* calibration method as previously used for the determination of the concentration of CB ( $\approx 40 \mu\text{M}$ ) in hippocampal granule cells (Muller et al., 2005) or of PV in DG basket cells ( $11.9 \pm 1.6 \mu\text{M}$ ) or in cerebellar basket cells ( $563 \pm 66 \mu\text{M}$ ) (Eggermann and Jonas, 2012). The particular biophysical properties of CR also acting as a slow-onset  $\text{Ca}^{2+}$  buffer are best appreciated from studies in *Xenopus* oocytes (Dargan et al., 2004). Photo-release of inositol 1,4,5 triphosphate ( $\text{IP}_3$ ) evokes  $\text{Ca}^{2+}$  signals that are differently modulated by endogenous or synthetic  $\text{Ca}^{2+}$  buffers (Dargan and Parker, 2003). In the presence of slow buffers such as PV or EGTA, global  $\text{Ca}^{2+}$  signals are fragmented into local  $\text{Ca}^{2+}$  puffs, resulting from  $\text{Ca}^{2+}$  release from discrete clusters of  $\text{IP}_3$  receptors, while low concentrations of fast buffers (CB, BAPTA) decrease the amplitude of  $\text{Ca}^{2+}$  signals and favor



“globalization” of spatially uniform  $\text{Ca}^{2+}$  signals, in particular, at high  $[\text{IP}_3]$ . Interestingly, puffs are observed in the presence of CR at low stimulation intensities, i.e., at low  $[\text{IP}_3]$ , an effect never occurring in the presence of CB or BAPTA. Thus, under conditions of small elevations in  $[\text{Ca}^{2+}]_i$  from resting  $\text{Ca}^{2+}$  levels, CR has properties of a slow  $\text{Ca}^{2+}$  buffer such as PV or EGTA.

## CALRETININ EXPRESSION IS LINKED TO NEURONAL DEVELOPMENT

The detailed analyses of temporal and spatial expression of CR in the brain is the major focus of this Frontiers series, has been summarized in several papers and reviews Arai et al. (1991); Jacobowitz and Winsky (1991); Hof et al. (1999); Barinka and Druga (2010) and is thus not covered in this mini-review. Recent findings on CR expression (often transient) and neurogenesis are briefly summarized. Olfactory receptor neurons are generated throughout lifetime and are characterized by a short period of CR expression just before these neurons are fully mature (Wei et al., 2013), yet the functional significance is currently unknown. Also mouse adult hippocampal neurogenesis, more precisely, the early postmitotic stage of dentate gyrus (DG) granule cell development is characterized by transient CR expression (Brandt et al., 2003). This stage coincides with onset of differentiation and absence of CR in the immature early postmitotic granule cells of  $\text{CR}^{-/-}$  mice (systematic name:  $\text{Calb2}^{\text{tm1Map}}$ ) “causes an early loss in proliferative capacity of the subgranular zone that is maintained into adult age, when it has a further impact on the migration/survival of newborn granule cells” (Todkar et al., 2012). Interestingly, when in WT mice newborn cells are functionally integrated in the DG granule cell network, CR expression stops and is changed to CB, the typical marker for adult DG granule cells. The functional consequences of this swap from CR to CB expression for granule cell physiology are currently unknown, as well as the mechanisms that lead to such a change. Nonetheless, it indicates  $\text{Ca}^{2+}$ -binding protein-specific functions that cannot be shared and/or substituted even by apparently very similar proteins such as CB and CR.

## WHAT IS THE PHYSIOLOGICAL ROLE OF CALRETININ?

As was previously reported for CB (Schmidt, 2012), CR certainly has more than one function, depending on various parameters including cell type (neurons vs. non-excitabile cells including tumor cells), stages of development (adult vs. developmental stages) and probably also different neuronal subtypes. For some proposed roles of CR, e.g., a role in neuroprotection, the proportion of reports, mostly obtained in correlative studies, in favor or against such a role is almost 50:50 [for more details, see (Schwaller, 2009, 2010)], clearly necessitating more studies directly addressing this putative function of CR. Here, just the most important results obtained in  $\text{CR}^{-/-}$  mice are summarized. The decreased LTP in the DG is thought to be the result of an increased excitatory drive from CR-depleted mossy cells onto hilar interneurons (Schurmans et al., 1997). Most findings on the function of CR are derived from studies in the cerebellum, where CR is expressed in cerebellar granule cells. Their increased excitability in the absence of CR (Gall et al., 2003) is linked to the altered firing properties (Cheron et al., 2000) and likely  $\text{Ca}^{2+}$

homeostasis of Purkinje cells and the emergence of cerebellar 160-Hz oscillations (Cheron et al., 2004) that result in impairment in motor coordination (Schiffmann et al., 1999), for more details, see Schwaller (2009). Thus, CR expression in granule cells appears necessary for correct computation that is crucial for the coding and storage of information in the cerebellum. Of note, there is currently no data available on CR's function in cortical interneurons, e.g., derived from  $\text{CR}^{-/-}$  mice. I am also not aware that anybody has attempted to manipulate CR expression levels of cortical interneurons, e.g., by shRNA and to investigate the functional consequences of CR down-regulation.

In CR-expressing mesothelial cells, CR down-regulation causes a  $G_1$  block and in mesothelioma-derived cells leads to apoptosis via strong activation of the intrinsic caspase 9-dependent pathway (Blum and Schwaller, 2013). A rather similar effect is also seen in CR-expressing colon cancer cells (Gander et al., 1996) indicating a role for CR in cell cycle regulation, proliferation, possibly differentiation and cell death. These findings are in line with transient CR expression during development, whether in the nervous system or in other tissues including mesenchymal tissue (Gangji et al., 1994). In summary, we have just started to unravel the likely many functions of CR in different settings and there are still plenty of interesting aspects on CR's function(s) to be discovered.

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## REFERENCES

- Alpar, A., Attems, J., Mulder, J., Hokfelt, T., and Harkany, T. (2012). The renaissance of  $\text{Ca}^{2+}$ -binding proteins in the nervous system: secretogin takes center stage. *Cell. Signal.* 24, 378–387. doi: 10.1016/j.celsig.2011.09.028
- Arai, R., Winsky, L., Arai, M., and Jacobowitz, D. M. (1991). Immunohistochemical localization of calretinin in the rat hindbrain. *J. Comp. Neurol.* 310, 21–44. doi: 10.1002/cne.903100105
- Arendt, O., Schwaller, B., Brown, E. B., Eilers, J., and Schmidt, H. (2013). Restricted diffusion of calretinin in cerebellar granule cell dendrites implies  $\text{Ca}^{2+}$ -dependent interactions via its EF-hand 5 domain. *J. Physiol.* 591, 3887–3899. doi: 10.1113/jphysiol.2013.256628
- Barinka, F., and Druga, R. (2010). Calretinin expression in the mammalian neocortex: a review. *Physiol. Res.* 59, 665–677.
- Bearzatto, B., Servais, L., Roussel, C., Gall, D., Baba-Aissa, F., Schurmans, S., et al. (2006). Targeted calretinin expression in granule cells of calretinin-null mice restores normal cerebellar functions. *FASEB J.* 20, 380–382. doi: 10.1096/fj.05-3785fje
- Billing-Marczak, K., Buzanska, L., Winsky, L., Nowotny, M., Rudka, T., Isaacs, K., et al. (2002). AP2-like cis element is required for calretinin gene promoter activity in cells of neuronal phenotype differentiated from multipotent human cell line DEV. *Biochim. Biophys. Acta* 1577, 412–420. doi: 10.1016/S0167-4781(02)00443-8
- Billing-Marczak, K., and Kuznicki, J. (1995). Calretinin -sensor or buffer-function still unclear. *Pol. J. Pharmacol.* 1173–1178. doi: 10.1267/ahc.06008
- Billing-Marczak, K., Przybyszewska, M., and Kuznicki, J. (1999). Measurements of  $[\text{Ca}^{2+}]_i$  using fura-2 in glioma C6 cells expressing calretinin with GFP as a marker of transfection: no  $\text{Ca}^{2+}$ -buffering provided by calretinin. *Biochim. Biophys. Acta* 1449, 169–177. doi: 10.1016/S0167-4889(99)00010-5
- Billing-Marczak, K., Zieminska, E., Lesniak, W., Lazarewicz, J. W., and Kuznicki, J. (2004). Calretinin gene promoter activity is differently regulated in neurons and cancer cells. Role of AP2-like cis element and zinc ions. *Biochim. Biophys. Acta* 1678, 14–21. doi: 10.1016/j.bbexp.2004.01.004

- Blum, W., and Schwaller, B. (2013). Calretinin is essential for mesothelioma cell growth/survival *in vitro*: a potential new target for malignant mesothelioma therapy? *Int. J. Cancer J. Int. du cancer* 133, 2077–2088. doi: 10.1002/ijc.28218
- Brandt, M. D., Jessberger, S., Steiner, B., Kronenberg, G., Reuter, K., Bick-Sander, A., et al. (2003). Transient calretinin expression defines early postmitotic step of neuronal differentiation in adult hippocampal neurogenesis of mice. *Mol. Cell. Neurosci.* 24, 603–613. doi: 10.1016/S1044-7431(03)00207-0
- Camp, A., Wijesinghe, J., and Calretinin, R. (2009). Modulator of neuronal excitability. *Int. J. Biochem. Cell Biol.* 41, 2118–2121. doi: 10.1016/j.biocel.2009.05.007
- Cheron, G., Gall, D., Servais, L., Dan, B., Maex, R., and Schiffmann, S. N. (2004). Inactivation of calcium-binding protein genes induces 160 Hz oscillations in the cerebellar cortex of alert mice. *J. Neurosci.* 24, 434–441. doi: 10.1523/JNEUROSCI.3197-03.2004
- Cheron, G., Schurmans, S., Lohof, A., d'Alcantara, P., Meyer, M., Draye, J. P., et al. (2000). Electrophysiological behavior of Purkinje cells and motor coordination in calretinin knock-out mice. *Prog. Brain Res.* 124, 299–308. doi: 10.1016/S0079-6123(00)24024-7
- Christel, C. J., Schär, R., Wang, S., Henzi, T., Kreiner, L., Grabs, D., et al. (2012). Calretinin regulates Ca<sup>2+</sup>-dependent inactivation and facilitation of CaV2.1 Ca<sup>2+</sup> channels through a direct interaction with the  $\alpha$ 12.1 subunit. *J. Biol. Chem.* 287, 39766–39775. doi: 10.1074/jbc.M112.406363
- Dargan, S. L., and Parker, I. (2003). Buffer kinetics shape the spatiotemporal patterns of IP3-evoked Ca<sup>2+</sup> signals. *J. Physiol.* 553, 775–788. doi: 10.1113/jphysiol.2003.054247
- Dargan, S. L., Schwaller, B., and Parker, I. (2004). Spatiotemporal patterning of IP3-mediated Ca<sup>2+</sup> signals in *Xenopus* oocytes by Ca<sup>2+</sup>-binding proteins. *J. Physiol.* 556, 447–461. doi: 10.1113/jphysiol.2003.059204
- Dong, G., Gross, K., Qiao, F., Ferguson, J., Callegari, E. A., Rezvani, K., et al. (2012). Calretinin interacts with huntingtin and reduces mutant huntingtin-caused cytotoxicity. *J. Neurochem.* 123, 437–446. doi: 10.1111/j.1471-4159.2012.07919.x
- D'Orlando, C., Fellay, B., Schwaller, B., Salicio, V., Bloc, A., Gotzos, V., et al. (2001). Calretinin and calbindin D-28k delay the onset of cell death after excitotoxic stimulation in transfected P19 cells. *Brain Res.* 909, 145–158. doi: 10.1016/S0006-8993(01)02671-3
- Edmonds, B., Reyes, R., Schwaller, B., and Roberts, W. M. (2000). Calretinin modifies presynaptic calcium signaling in frog saccular hair cells. *Nat. Neurosci.* 3, 786–790. doi: 10.1038/77687
- Eggermann, E., and Jonas, P. (2012). How the 'slow' Ca<sup>2+</sup> buffer parvalbumin affects transmitter release in nanodomain-coupling regimes. *Nat. Neurosci.* 15, 20–22. doi: 10.1038/nn.3002
- Faas, G. C., Schwaller, B., Vergara, J. L., and Mody, I. (2007). Resolving the fast kinetics of cooperative binding: Ca<sup>2+</sup> buffering by calretinin. *PLoS Biol.* 5:e311 doi: 10.1371/journal.pbio.0050311
- Gall, D., Roussel, C., Susa, I., D'Angelo, E., Rossi, P., Bearzatto, B., et al. (2003). Altered neuronal excitability in cerebellar granule cells of mice lacking calretinin. *J. Neurosci.* 23, 9320–9327.
- Gander, J. C., Gotzos, V., Fellay, B., and Schwaller, B. (1996). Inhibition of the proliferative cycle and apoptotic events in WiDr cells after down-regulation of the calcium-binding protein calretinin using antisense oligodeoxynucleotides. *Exp. Cell Res.* 225, 399–410. doi: 10.1006/excr.1996.0191
- Gander, J.-C., Bustos-Castillo, M., Stüber, D., Hunziker, W., Celio, M. R., and Schwaller, B. (1996). The calcium-binding protein calretinin-22k, an alternative splicing product of the calretinin gene is expressed in several colon adenocarcinoma cell lines. *Cell Calcium* 20, 63–72. doi: 10.1016/S0143-4160(96)90051-2
- Gangji, V., Bastianelli, E., Rooze, M., and Pochet, R. (1994). Transient Calretinin Expression during Intervertebral Disc Formation of the Chick Embryo. *Dev. Growth Differ.* 36, 621–628. doi: 10.1111/j.1440-169X.1994.00621.x
- Hack, N. J., Wride, M. C., Charters, K. M., Kater, S. B., and Parks, T. N. (2000). Developmental changes in the subcellular localization of calretinin. *The Journal of neurosci.* 20, RC67.
- Hackney, C. M., Mahendrasingam, S., Penn, A., and Fettiplace, R. (2005). The concentrations of calcium buffering proteins in mammalian cochlear hair cells. *J. Neurosci.* 25, 7867–75. doi: 10.1523/JNEUROSCI.1196-05.2005
- Haner, K., Henzi, T., Pfefferli, M., Kunzli, E., Salicio, V., and Schwaller, B. (2010). A bipartite butyrate-responsive element in the human calretinin (CALB2) promoter acts as a repressor in colon carcinoma cells but not in mesothelioma cells. *J Cell Biochem.* 109, 519–531. doi: 10.1002/jcb.22429
- Henzi, T., Blum, W. V., Pfefferli, M., Kaweck, T. J., Salicio, V., and Schwaller, B. (2009). SV40-induced expression of calretinin protects mesothelial cells from asbestos cytotoxicity and may be a key factor contributing to mesothelioma pathogenesis. *Am. J. Pathol.* 174, 2324–2336. doi: 10.2353/ajpath.2009.080352
- Hof, P. R., Glezer, I. I., Conde, F., Flagg, R. A., Rubin, M. B., Nimchinsky, E. A., et al. (1999). Cellular distribution of the calcium-binding proteins parvalbumin, calbindin, and calretinin in the neocortex of mammals: phylogenetic and developmental patterns. *J. Chem. Neuroanat.* 16, 77–116. doi: 10.1016/S0891-0618(98)00065-9
- Jacobowitz, D. M., and Winsky, L. (1991). Immunocytochemical localization of calretinin in the forebrain of the rat. *J. Comp. Neurol.* 304, 198–218. doi: 10.1002/cne.903040205
- Kojetin, D. J., Venters, R. A., Kordys, D. R., Thompson, R. J., Kumar, R., and Cavanagh, J. (2006). Structure, binding interface and hydrophobic transitions of Ca<sup>2+</sup>-loaded calbindin-D(28K). *Nat. Struct. Mol. Biol.* 13, 641–647. doi: 10.1038/nsmb1112
- Kuznicki, J., Isaacs, K. R., and Jacobowitz, D. M. (1996). The expression of calretinin in transfected PC12 cells provides no protection against Ca<sup>2+</sup>-overload or trophic factor deprivation. *Biochim. Biophys. Acta* 1313, 194–200. doi: 10.1016/0167-4889(96)00089-4
- Kuznicki, J., Wang, T.-C. L., Martin, B. M., Winsky, L., and Jacobowitz, D. M. (1995). Localization of Ca<sup>2+</sup>-dependent conformational changes of calretinin by limited tryptic proteolysis. *Biochem. J.* 308, 607–619.
- Marilley, D., Schwaller, B. (2000). Association between the calcium-binding protein calretinin and cytoskeletal components in the human colon adenocarcinoma cell line WiDr. *Exp Cell Res.* 259, 12–22. doi: 10.1006/excr.2000.4942
- Marilley, D., Vonlanthen, S., Gioria, A., and Schwaller, B. (2001). Calretinin and calretinin-22k increase resistance towards sodium butyrate-induced differentiation in CaCo-2 colon adenocarcinoma cells. *Exp Cell Res.* 268, 93–103. doi: 10.1006/excr.2001.5261
- Muller, A., Kukley, M., Stausberg, P., Beck, H., Muller, W., and Dietrich, D. (2005). Endogenous Ca<sup>2+</sup> buffer concentration and Ca<sup>2+</sup> microdomains in hippocampal neurons. *J. Neurosci.* 25, 558–565. doi: 10.1523/JNEUROSCI.3799-04.2005
- Palczewska, M., Groves, P., Ambrus, A., Kaleta, A., Kover, K. E., Batta, G., et al. (2001). Structural and biochemical characterization of neuronal calretinin domain I-II (residues 1-100). Comparison to homologous calbindin D28k domain I-II (residues 1-93). *Eur. J. Biochem.* 268, 6229–6237. doi: 10.1046/j.0014-2956.2001.02575.x
- Pecze, L., Blum, W., and Schwaller, B. (2013). Mechanism of capsaicin receptor TRPV1-mediated toxicity in pain-sensing neurons focusing on the effects of Na<sup>+</sup>/Ca<sup>2+</sup> fluxes and the Ca<sup>2+</sup>-binding protein calretinin. *Biochim. Biophys. Acta* 1833, 1680–1691. doi: 10.1016/j.bbamcr.2012.08.018
- Reifegerste, R., Grimm, S., Albert, S., Lipski, N., Heimbeck, G., Hofbauer, A., et al. (1993). An invertebrate calcium-binding protein of the calbindin subfamily: protein structure, genomic organization, and expression pattern of the calbindin-32 Gene of *Drosophila*. *J. Neurosci.* 13, 2186–2198.
- Rogers, J. H. (1987). Calretinin: a gene for a novel calcium-binding protein expressed principally in neurons. *J. Cell Biol.* 105, 1343–1353. doi: 10.1083/jcb.105.3.1343
- Roussel, C., Erneux, T., Schiffmann, S. N., and Gall, D. (2006). Modulation of neuronal excitability by intracellular calcium buffering: from spiking to bursting. *Cell Calcium* 39, 455–466. doi: 10.1016/j.ceca.2006.01.004
- Saftenu, E. E. (2012). Effects of calretinin on Ca<sup>2+</sup> signals in cerebellar granule cells: implications of cooperative Ca<sup>2+</sup> binding. *Cerebellum* 11, 102–120. doi: 10.1007/s12311-011-0263-4
- Schiffmann, S. N., Cheron, G., Lohof, A., d'Alcantara, P., Meyer, M., Parmentier, M., et al. (1999). Impaired motor coordination and Purkinje cell excitability in mice lacking calretinin. *Proc. Natl. Acad. Sci. U.S.A.* 96, 5257–5262. doi: 10.1073/pnas.96.9.5257
- Schmidt, H. (2012). Three functional facets of calbindin D-28k. *Front. Mol. Neurosci.* 5:25. doi: 10.3389/fnmol.2012.00025
- Schmidt, H., Brachtendorf, S., Arendt, O., Hallermann, S., Ishiyama, S., Bornschein, G., et al. (2013). Nanodomain coupling at an excitatory cortical synapse. *Curr. Biol.* 23, 244–249. doi: 10.1016/j.cub.2012.12.007
- Schurmans, S., Schiffmann, S. N., Gurden, H., Lemaire, M., Lipp, H.-P., Schwam, V., et al. (1997). Impaired LTP induction in the dentate gyrus of calretinin-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 94, 10415–10420. doi: 10.1073/pnas.94.19.10415

- Schwaller, B. (2009). The continuing disappearance of “pure”  $\text{Ca}^{2+}$  buffers. *Cell Mol. Life Sci.* 66, 275–300. doi: 10.1007/s00018-008-8564-6
- Schwaller, B. (2010). Cytosolic  $\text{Ca}^{2+}$  buffers. *Cold Spring Harb. Perspect. Biol.* 2:a004051. doi: 10.1101/cshperspect.a004051
- Schwaller, B. (2012). The Regulation of a Cell's  $\text{Ca}^{2+}$  Signaling Toolkit: The  $\text{Ca}^{2+}$  Homeostasome. *Adv. Exp. Med. Biol.* 740, 1–25. doi: 10.1007/978-94-007-2888-2\_1
- Schwaller, B., and Herrmann, B. (1997). Regulated redistribution of calretinins in WiDr cells. *Cell Death Differ.* 4, 325–333. doi: 10.1038/sj.cdd.4400240
- Schwaller, B., Celio, M. R., and Hunziker, W. (1995). Alternative splicing of calretinin mRNA leads to different forms of calretinin. *Eur. J. Biochem.* 230, 424–430. doi: 10.1111/j.1432-1033.1995.0424h.x
- Schwaller, B., Durussel, I., Jermann, D., Herrmann, B., and Cox, J. A. (1997). Comparison of the  $\text{Ca}^{2+}$ -binding properties of human recombinant calretinin-22k and calretinin. *J. Biol. Chem.* 272, 29663–29671. doi: 10.1074/jbc.272.47.29663
- Schwaller, B., Meyer-Monard, S., Gander, J.-C., Pugin, P., Celio, M. R., and Ludwig, C. (1998). The calcium-binding protein calretinin-22k is detectable in the serum and specific cells of cancer patients. *Anticancer Res.* 18, 3361–3367.
- Stevens, J., and Rogers, J. H. (1997). Chick calretinin: purification, composition, and metal binding activity of native and recombinant forms. *Protein Expr. Purif.* 9, 171–181. doi: 10.1006/prep.1996.0677
- Strauss, K. I., Kuznicki, J., Winsky, L., Kawagoe, J. I., Hammer, M., and Jacobowitz, D. M. (1997). The mouse calretinin gene promoter region: structural and functional components. *Brain Res. Mol. Brain Res.* 49, 175–187. doi: 10.1016/S0169-328X(97)00143-5
- Todkar, K., Scotti, A. L., and Schwaller, B. (2012). Absence of the calcium-binding protein calretinin, not of calbindin D-28k, causes a permanent impairment of murine adult hippocampal neurogenesis. *Front. Mol. Neurosci.* 5:56. doi: 10.3389/fnmol.2012.00056
- Wei, H., Lang, M. F., and Jiang, X. (2013). Calretinin is expressed in the intermediate cells during olfactory receptor neuron development. *Neurosci. Lett.* 542, 42–46. doi: 10.1016/j.neulet.2013.03.022
- Winsky, L., and Kuznicki, J. (1995). Distribution of calretinin, calbindin D28k and parvalbumin in subcellular fractions of rat cerebellum: effects of calcium. *J. Neurochem.* 65, 381–388. doi: 10.1046/j.1471-4159.1995.65010381.x
- Wisniewska, M. B., Nagalski, A., Dabrowski, M., Misztal, K., and Kuznicki, J. (2012). Novel beta-catenin target genes identified in thalamic neurons encode modulators of neuronal excitability. *BMC Genomics* 13:635. doi: 10.1186/1471-2164-13-635
- Zimmermann, L., and Schwaller, B. (2002). Monoclonal antibodies recognizing epitopes of calretinins: dependence on  $\text{Ca}^{2+}$ -binding status and differences in antigen accessibility in colon cancer cells. *Cell Calcium* 31, 13–25. doi: 10.1054/ceca.2001.0255

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