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Review article

# PMCA2 pump mutations and hereditary deafness

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ABSTRACT

Hair cells of the inner ear detect sound stimuli, inertial or gravitational forces by deflection of their apical stereocilia. A small number of stereociliary cation-selective mechanotransduction (MET) channels admit $K^+$ and $Ca^{2+}$ ions into the cytoplasm promoting hair cell membrane depolarization and, consequently, neurotransmitter release at the cell basolateral pole. $Ca^{2+}$ influx into the stereocilia compartment is counteracted by the unusual *w/a* splicing variant of plasma-membrane calcium-pump isoform 2 (PMCA2) which, unlike other PMCA2 variants, increases only marginally its activity in response to a rapid variation of the cytoplasmic free $Ca^{2+}$ concentration ($[Ca^{2+}]_c$). Missense mutations of PMCA2*w/a* cause deafness and loss of balance in humans. Mouse models in which the pump is genetically ablated or mutated show hearing and balance impairment, which correlates with defects in homeostatic regulation of stereociliary $[Ca^{2+}]_c$, decreased sensitivity of mechanotransduction channels to hair bundle displacement and progressive degeneration of the organ of Corti. These results highlight a critical role played by the PMCA2*w/a* pump in the control of hair cell function and survival, and provide mechanistic insight into the etiology of deafness and vestibular disorders.

## 1. Introduction

The plasma membrane $Ca^{2+}$-ATPase (PMCA) pump, together with the $Na^+/Ca^{2+}$ exchanger (NCX), is the main $Ca^{2+}$ exporting system of eukaryotic cells, and maintains $Ca^{2+}$ ions at the low concentration demanded by their signaling function in the cytoplasm [15]. In contrast to the NCX, which is better suited for removing quickly large amounts of $Ca^{2+}$, the PMCA pump operates with high $Ca^{2+}$ affinity and low transport capacity, with a 1:1 $Ca^{2+}$/ATP stoichiometry. The PMCA pump can operate both in the nanomolar and in the micromolar $Ca^{2+}$ range by a regulatory-domain complex activated by calmodulin (CaM), which binds with different affinity two different sites in the C-terminal domain, where the less sensitive binding site is located downstream of the canonical site [65]. Two basic PMCA isoforms can be found in all tissues (PMCA1 and 4), while the other two (PMCA2 and 3) are expressed mainly in specialized tissues, such as muscle and brain [15,33]. In particular, the PMCA2 pump is expressed at high levels in vestibular and outer hair cell stereocilia and apical membranes, and at moderate levels in inner hair cell stereocilia and in the spiral ganglion [16,22,24,31,32]. This pump isoform has properties that set it apart from all other PMCAs [11]: it has very high affinity for CaM, yet, its activity is only modestly activated by it [11,35].

Unlike the other three PMCA basic isoforms, the PMCA2 pump has peculiarly high activity in the absence of CaM, i.e., it pumps $Ca^{2+}$ out of cells at a relatively high constant rate. Splicing involves the insertion of one or three exons at site A, in the first cytosolic loop of the pump, and one or two exons at site C in the C-terminal domain [40] (Fig. 1).

The A-site insertions are in frame, creating variant *w* when 3 exons are inserted or the normal variant *z* without inserts. The expression of the splice variant PMCA2*x* is induced only by a transient rise of intracellular $Ca^{2+}$, suggesting that signaling pathways involving serine/threonine phosphorylation event are a necessary intermediate step to a change in alternative splicing in the nucleus [62,74]. Insertion at site C creates instead a novel stop codon, leading to truncation of the pump in variant *a*, variant *b* being the normal full-length pump and *c* a C-terminal shortened version due to a change in the reading frame. The C-site insertions eliminate about half of the CaM binding domain, those at site A occur next to a domain that binds activator acidic phospholipids, which, however, also bind the CaM binding domain [13]. As expected, the C insertions lower the affinity of the PMCA2 pump for CaM [26], but do not compromise the activation by acidic phospholipids, which is alternative to that by CaM [51].

The C-terminally truncated PMCA2*a* pump is the only isoform detected in the stereocilia of inner ear hair cells [24], whereas PMCA1*b*

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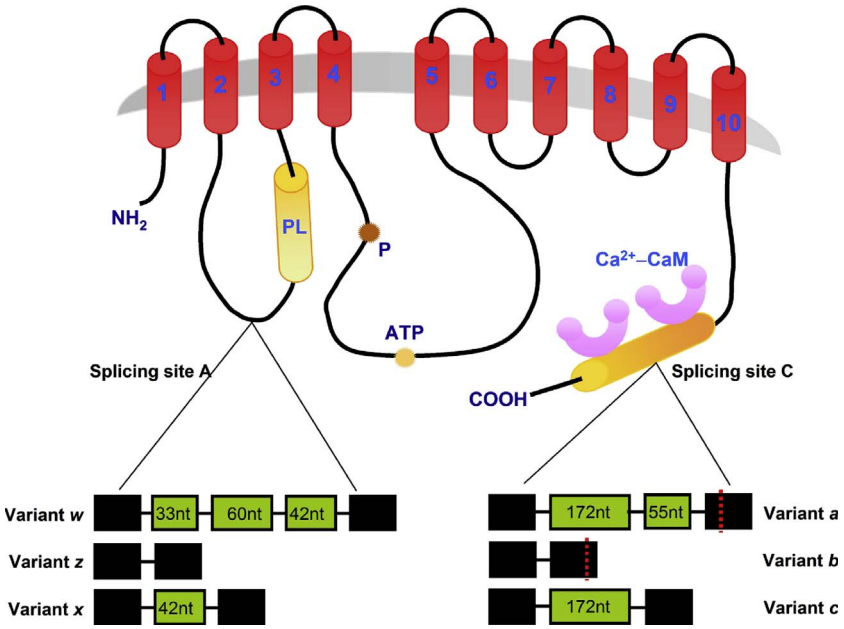


**Fig. 1.** PMCA2 topology and splicing variants. The pump is powered by the hydrolysis of adenosine triphosphate (ATP), with a stoichiometry of one $Ca^{2+}$ ion removed for each molecule of ATP hydrolysed. PL = phospholipid binding domain; P = location of the aspartyl-phosphate formation. Alternative splicing of human PMCA2 pump at site A located between transmembrane domains 2 and 3 generates variants *w*, *z*, x, or *y*. Variants *a*, *b*, or *c* at site C are generated by alternative splicing in the C-terminal region. Constitutively spliced exons are indicated as black boxes, alternatively inserted exons are shown in green (nt = number of nucleotides); the positions of the translation stop codons for each variant at site C are indicated by red dashed lines. Splice site C lies within a domain (yellow cylinder) which can bind one or two CaM molecules, depending on the splice variant. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

prevails in their basolateral membrane. Recent work has also shown that the PMCA2 pump is spliced at site A in the stereocilia of cochlear outer hair cells (OHCs, [44]), therefore yielding the *w/a* variant [36]. Mutations of the *ATP2b2* gene, coding for the PMCA2 pump, have been causally linked to hereditary deafness, loss of balance and ataxia in humans [29,57] and mice [7,39,50,61,63,64,66]. In some cases, these mutations lead to the truncation of the protein product, and to its eventual disappearance from the stereocilia of mouse hair cells. Other mutations, which do not compromise the reading frame of the gene and are thus compatible with the expression of the entire PMCA2*w/a* variant of the pump, affect residues that are highly conserved in all PMCA isoforms across species and in other P-type pumps.

Here we recapitulate work carried out in our laboratory to analyze the functional consequences of PMCA2*w/a* mutations in expression systems as well as in vestibular and cochlear hair cells derived from mutant mice, in collaboration with research teams led by Ernesto Carafoli, Marisa Brini, Paolo Gasparini, Karen Steel, Steve Brown and the late Edoardo Arslan. A limited number of key results generated by other laboratories are also reviewed, aiming to convey a broader message to the reader.

## 2. Inner ear mechanotransduction

The relationship between auditory and vestibular function and PMCA2*w/a* pump activity resides in the mechanotransduction process performed by sensory hair cells [14]. Current consensus holds that deflection of the stereociliary bundle in the direction of the taller stereocilia increases tension in the tip link, a filament stretched between adjacent stereocilia [27]. This mechanical stimulus is conveyed to MET channels, which are located at the stereocilia top, and open to allow influx of cations into the cell [19]. From embryonic development to first postnatal (P) days, also “unconventional” mechanically sensitive ion channels containing the Piezo2 protein are found in the apical plasma membrane of cochlear hair cells, but their physiological function is still debated [5,72]. The apical surface of hair cells is bathed in the endolymph, which is rich in $K^+$ but low in $Na^+$ and $Ca^{2+}$ [1], thus $K^+$ carries most the transduction current. However, due to the high permeability of MET channels to $Ca^{2+}$, its influx is significant despite the low $Ca^{2+}$ levels of the endolymph, which are much lower than those of other extracellular fluids [17,23,37,46,53]: 20–23 μM in the rodent cochlea [8,69], 200–250 μM in the vestibular system, possibly due to the presence there of calcium carbonate crystals [18,49].

### 2.1. PMCA2 in hair cells

Irrespective of the hair cell type, $Ca^{2+}$ entering through MET channels is sequestered by buffers in the stereocilia [34,56] and then shuttled back to the endolymph by the PMCA2*w/a* pump [45,73]. Various laboratories estimated the density of this pump in stereociliary membrane at 2000–2200/μm$^2$ [16,21,55,71,73], from which a figure of about 200 ions/s per pump has been inferred for the extrusion rate [16]. PMCA2*w/a* action is thought to increase $Ca^{2+}$ in the immediate proximity of the hair bundle [73], possibly with the complicity of the acellular structures overlying the hair cells in the cochlea [60] and, particularly, in the vestibular system [49], where the pump would also contribute to the formation and maintenance of the otoconia [39].

### 2.2. Mutations in humans

Ablation or missense mutations of the PMCA2 $Ca^{2+}$ pump of the stereocilia cause deafness and loss of balance [30]. Therefore $Ca^{2+}$ concentration in endolymph is expected to fall, causing an alteration of the mechanotransduction process. This may provide a clue as to why, both in humans [29,57] and mice [52,68], PMCA2 mutations potentiated the deafness phenotype induced by co-existing mutations of cadherin-23 (*Usher syndrome type* 1D, OMIM #61067), a single pass membrane $Ca^{2+}$ binding protein that is a component of the tip link in hair cell stereocilia (Fig. 2C). Gene therapy recently restored auditory and vestibular function to near wt levels in a mouse model of Usher syndrome type 1c, suggesting that biological therapies to treat deafness may be suitable for translation to humans with genetic inner ear disorders [54].

The cooperation of the cadherin-23 and the PMCA2 pump is evidently critical in the molecular events that ultimately permit the neural encoding of the acoustic signal. It is thus easy to appreciate the importance of their mutations in the generation of a hearing loss phenotype. Homozygous cadherin-23 mutations that impair the ability of the protein to bind $Ca^{2+}$, as detected in one of the human families described in [57], may be sufficient to disrupt the opening properties of the MET channels, generating hearing loss, which is then only exacerbated by the concomitant PMCA2 pump mutation. On the other hand, homozygous PMCA2 pump mutations, as found in the *Oblivion*