

Chloride accumulation in endosomes and lysosomes: facts checked in mice

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Loss of the CIC-3 chloride/proton exchanger found in intracellular compartments leads to marked neurodegeneration. New genetic work by Weinert *et al* (2020) now shows that selective impairment of CIC-3's ion exchange activity is sufficient to elicit this severe phenotype *in vivo*. CIC-3 cooperates with the closely related CIC-4 in protecting endolysosomal chloride balance and neuronal integrity.

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See also: S Weinert *et al* (May 2020)

Endosomes and lysosomes are compartments with critical functions in nutrient uptake, hormone signaling, detoxification, and the recycling of metabolites or cellular building blocks. The ion composition of the aqueous phase inside these compartments is critical to the broad spectrum of biochemical reactions that occur in both of them—from uncoupling receptors and their ligands to the activity of many different enzymes. For decades, the concentration of protons, or pH, inside endosomes and lysosomes has received intense attention. While any biochemist would agree that pH is one of the key parameters for optimal enzyme function, it is well known that the correct concentration of other ions can be an equally important factor. In the past decade, it has emerged that the chloride ion is indeed critical for endosomal and lysosomal function (Novarino *et al*, 2010; Weinert *et al*, 2010).

The accumulation of protons is an electrogenic (potential-building) process. Hence, it is more effective when ions of the opposite charge are allowed to accompany the protons inside the vesicular compartment. Simply speaking—if the pH is all that

counts (in contrast to the proton electromotive force that is harnessed by mitochondria to make ATP), then send any negatively charged ion in parallel to the protons and you can accumulate them to a higher concentration, i.e., acidify the vesicle more efficiently. This mechanism is called “chloride-dependent shunt”. For the longest time, Ockham’s razor seemed to suggest very plausibly that endosomal or lysosomal chloride was just this, any negatively charged ion compensating for the protons’ positive charge.

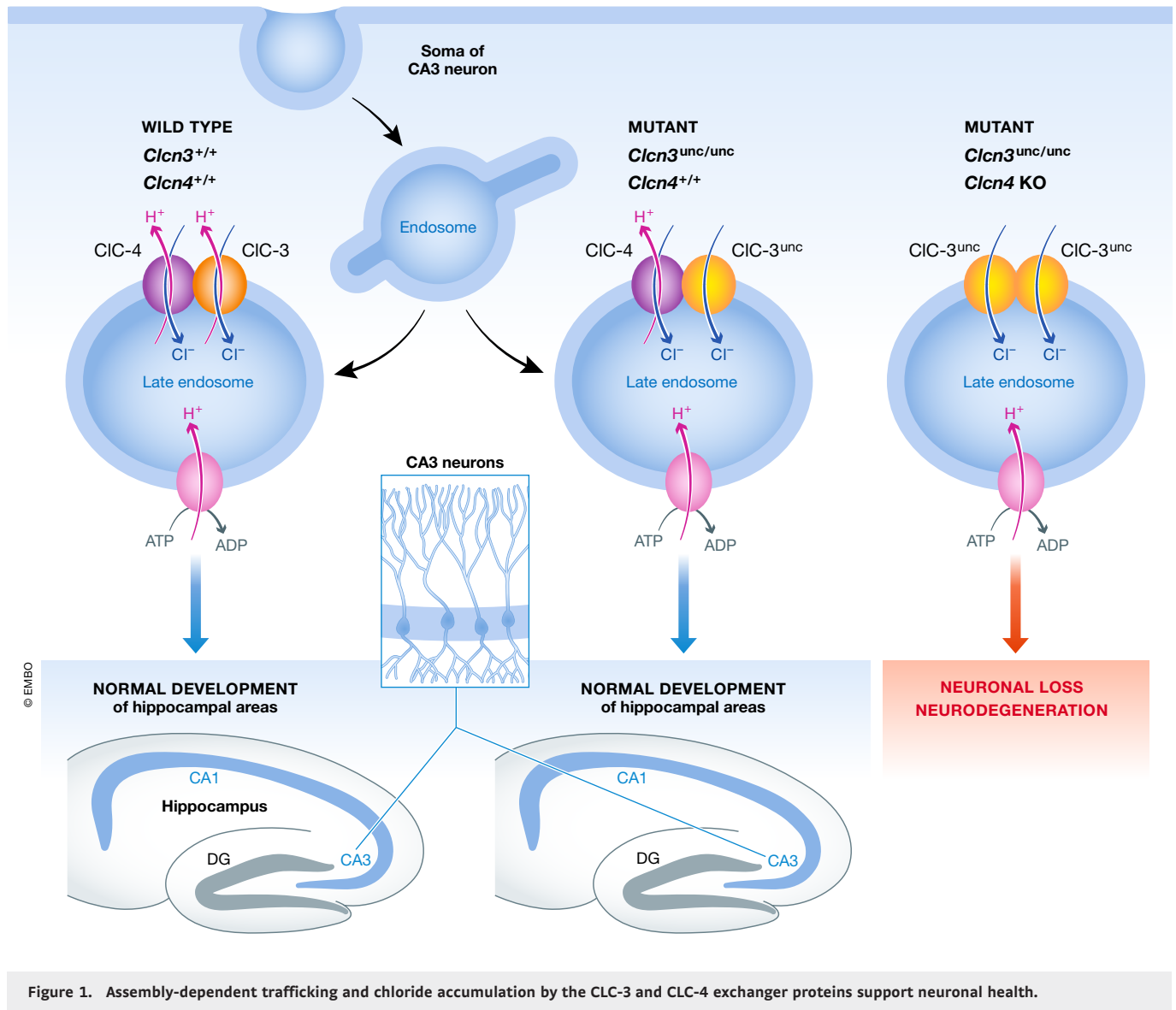
It seemed unnecessary to challenge this concept and has, indeed, proven experimentally difficult to do so. The impulse to revisit a seemingly solved problem came from a seminal discovery by Accardi and Miller (2004), their amazing demonstration that prokaryotic CLC homologues act as secondary active transporters and are able to use the energy stored in one ion’s gradient to move a second one against its electrochemical potential (secondary active transport). Prior to this discovery, CLC proteins were thought to be chloride channels, passively allowing chloride ions to move in the energetically favored direction.

The unexpected discovery raised many questions regarding the biophysical transport mechanism and the physiological relevance of animal or plant intracellular membrane proteins of the CLC family, such as mammalian CIC-3, CIC-4, CIC-5, CIC-6, and CIC-7. Indeed, these proteins were found to function as $2\text{Cl}^-/\text{H}^+$ exchangers, too. This made the hypothesis that chloride “just” provides negative charge mechanistically more complicated but still tenable since it was observed that losing such transporters did in some cases impair proper acidification of endosomes (Günther *et al*,

2003) or the osteoclast resorption lacuna, sometimes considered to be an “extracellular lysosome” (Kornak *et al*, 2001). So, why look any further and wonder about a putative role of chloride beyond being negative? In science, as in real life, the most obvious explanation is not always the correct one or at least not the full truth. To echo George Gershwin, “It ain’t necessarily so”.

The normal lysosomal pH of mice lacking lysosomal CIC-7 or its β -subunit Ostml1 (Kasper *et al*, 2005; Lange *et al*, 2006), which display severe lysosomal storage disease, suggested that CLC exchangers not only serve as chloride-dependent shunts. Addressing the question if coupled transport activity is per se physiologically relevant, the Jentsch Lab has exploited the discovery of uncoupling CLC mutations (Accardi & Miller, 2004) that convert the exchangers into a passively conducting, chloride-permeable pore: They embarked on the construction of a series of transgenic mouse models expressing uncoupled intracellular CLC proteins and started a careful and systematic comparison to the corresponding knockout mouse models. This strategy is elegant not only because it segregates the physiological relevance of two biophysically distinct transport modes—passive chloride leak as opposed to proton gradient-driven chloride accumulation. It also segregates the effects of losing the best-characterized and presumably central function of a protein from the effect of losing the protein per se, with all its protein–protein interactions, regulation, or unknown functions.

The work of Weinert *et al* (2020) now reveals just how hard one had to look to tackle the relevance of the exchange mode to the neuroprotective capacity of CIC-3. They replaced CIC-3 with the uncoupled



variant of the protein $CLC-3^{unc/unc}$. $Clcn3^{-/-}$ knockout mice lose their hippocampus and turn blind due to retinal degeneration (Stobrawa *et al*, 2001). Surprisingly, and in contrast to the work on $CLC-5$ and $CLC-7$ where the replacement by the uncoupled transporter phenocopies the knockout (Novarino *et al*, 2010; Weinert *et al*, 2010), this was not the case in $Clcn3^{unc/unc}$ mice, which turned out to be unexpectedly normal (Fig 1).

CLC membrane proteins are obligatory dimers with an ion-conducting pathway in each monomer. The overlapping expression profiles of several CLC proteins raise the possibility of heterodimerization, which has been demonstrated for some combinations

but so far not shown to be essential for any CLC transporter's cellular function. Biochemical investigation of the steady-state levels and glycosylation status of $CLC-4$, a close homologue of $CLC-3$ also robustly expressed in neurons, enabled Weinert *et al* (2020) to reveal that $CLC-4$ has to co-assemble with $CLC-3$ to leave the early secretory pathway and to provide its ion transport activity to the right cellular compartment in the endolysosomal system. Indeed, mice expressing $Clcn3^{unc/unc}$ but lacking $Clcn4$ show severe neurodegeneration (Fig 1). As with the intracellular $CLC-5$ and $CLC-7$, the exchanger transport mode, and hence the capacity to accumulate chloride inside vesicles, is critical to $CLC-3$'s physiological function. This is

masked by co-assembly with $CLC-4$ and only revealed in a mouse model combining the knock-in of uncoupled $CLC-3$ with a knockout of the $Clcn4$ gene (which does not give rise to a severe phenotype illustrating that $CLC-3$ can fulfill its basic function without $CLC-4$).

In conclusion, this work unequivocally shows the central role of CLC-mediated chloride/proton exchange and hence vesicular chloride accumulation in the neuroprotective function of $CLC-3$. It provides a clear example of assembly-dependent trafficking of a multimeric membrane protein and thereby illustrates that forward protein transport along the secretory pathway is anything but default. Like all intriguing scientific work, the article by Weinert *et al*

(2020) opens new questions: Why is the ClC-4 protein so strictly attended by ClC-3? Is there a specific functional or regulatory capacity endowed by ClC-4 to ClC-3/ClC-4 heterodimers? Genetic links to human brain disorders suggest that this may be the case. One can hope that this and related questions around the biochemical function of chloride ions inside endosomal or lysosomal compartments will be pursued by a similarly rich portfolio of cutting-edge approaches in gene targeting, biochemistry, histology, cell biology, and physiology to elucidate even unexpected facts.

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