RESEARCH HIGHLIGHT

Identity revealed for a long-sought ER anion channel

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Ca^{2+} release from the endoplasmic reticulum (ER) requires flux of counterions. In a recent paper published in *Cell Research*, Guo and colleagues, using a powerful combinatorial approach, show that CLCC1 is the long-sought mediator of Cl^- efflux from the ER that counteracts Ca^{2+} release and that its loss causes ER stress and contributes to ALS pathology.

The endoplasmic reticulum (ER) is a network of intracellular membranes found in all eukaryotic cells except for mature red blood cells. The ER has many functions. It is the site of biosynthesis and folding of transmembrane and secreted proteins, it is where protein modifications such as glycosylation and cleavage occur, and it is the site of steroid hormone and phospholipid synthesis. The ER is also a regulator of intracellular Ca^{2+} concentration. Ca^{2+} is a key ion in many physiological functions from excitability, secretion, and contraction to regulation of the function of many cytosolic and membrane proteins. Thus, precise regulation of Ca^{2+} homeostasis has important physiological consequences, and its dysregulation can lead to disease.

Ca^{2+} is released from the ER via ryanodine receptors (RyRs) and inositol 1,4,5-trisphosphate receptors (IP3Rs) and is taken up via the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA). The movement of Ca^{2+} across the ER membrane is electrogenic. For example, Ca^{2+} release from the ER tends to accumulate negative charges in the *trans* compartment, which impedes further Ca^{2+} release. Thus, mediators for the flux of counterions must exist to neutralize this charge accumulation.

K^+ and Cl^- have been proposed to function as counterions. Biochemical and electrophysiological approaches on ER membranes have identified the Trimeric Intracellular Cation channels (TRIC) as the major mediators of K^+ influx that counteracts Ca^{2+} release.[1] In addition, other K^+ channels, including the small-conductance Ca^{2+}-activated K^+ (KCa) channels (SK channels) as well as the RyR and IP3 receptors with intrinsic K^+ conductance, have been suggested to contribute to ER K^+ influx[2–4] (Fig. 1).

Mediators of Cl^- efflux from the ER have been more elusive, even though various Cl^- channels have been localized to the ER membrane. For example, large-conductance Cl^- channels have been described in ER vesicles.[5] However, these may represent peptide-transporting channels not involved in Ca^{2+} homeostasis. Furthermore, chloride intracellular channels (CLICs) have been proposed to function as ER channels as well. However, the exitance of CLIC channels in both membrane-inserted and soluble forms is a confounding factor in the localization of these channels to intracellular membranes.[6] The only two CLIC channels for which intracellular membrane localization is more strongly supported are CLIC5B and CLIC4. However, neither of these two channels is in the ER membrane.[7,8] Bestrophins have been also proposed to release Cl^- from the ER.[9] However, the evidence, in this case, is similarly weak or at least points to additional contributors to Cl^- flux.

In this issue of *Cell Research*, using a combination of powerful and complementary techniques, Guo and colleagues provide evidence that Chloride Channel CLIC Like 1 (CLCC1), an ER-resident protein cloned more than 20 years ago,[10] is a mediator of Cl^- efflux in the ER.[11] In the first set of experiments, Guo and colleagues use immunocytochemistry, biochemical approaches, and electrophysiology to show that mouse and/or human CLCC1 colocalize with ER protein calnexin, form a protein complex composed of at least two subunits, and generate single channel and microscopic anion currents when reconstituted in the lipid bilayer. The authors also show that CLCC1 current is inhibited by Ca^{2+} in the *trans* compartment of the lipid bilayer, which corresponds to the ER lumen, and further identify two aspartic residues in the luminal N-terminus that are responsible for Ca^{2+} inhibition (Fig. 1). This result is consistent with the function of a postulated charge neutralizing Cl^- efflux channel that needs to be engaged when luminal ER Ca^{2+} concentration drops.

Using two newly developed ER Cl^- and K^+ sensors called RaMorideER and RaMssiumER, and shRNA-mediated knockdown of *CLCC1*, the authors proceed to show that luminal concentration of these ions is increased in cells lacking CLCC1. This result is consistent with the idea that CLCC1 mediates Cl^- efflux from the ER and further shows that K^+ influx is enhanced by loss of CLCC1. This may represent a compensatory mechanism, perhaps mediated by negative charge accumulation, that limits the negative effects of loss of CLCC1. Finally, the authors demonstrate altered Ca^{2+} oscillations induced by ATP in HEK cells treated with *CLCC1* shRNA and by caffeine in the cardiomyocytes of mice harboring an hypomorphic *CLCC1* mutation.

As mentioned earlier, the ER is where secreted and membrane proteins are folded. Disturbance of ER protein folding due to either external stress or malfunction of ER homeostasis triggers ER stress and activation of the unfolded protein response (UPR). The UPR consequently engages signaling pathways that try to compensate for the damage. Guo and colleagues notice that the volume of the ER is enlarged in cells in which *CLCC1* is knocked down and in cells expressing CLCC1(K298A), a mutant identified in this study that does not respond to PIP2 with enhanced channel activity (Fig. 1). These results suggest that reduction of function of CLCC1 may cause ER stress. To test this idea, the authors monitor BiP and ubiquitin staining, both hallmarks of ER stress, in cerebellar granule cells, dentate gyrus neurons, and motoneurons of K298A knock-in mice. They find enhanced ER stress in all these neuronal types as well as prominent degeneration of

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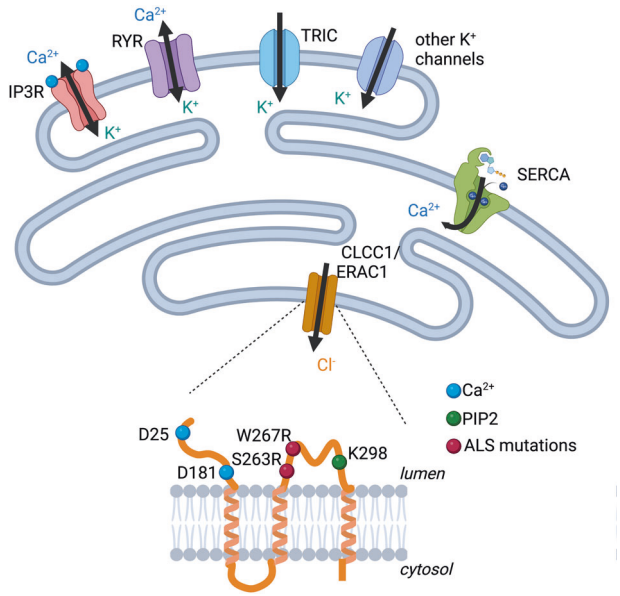

Fig. 1 CLCC1 anion channel mediates ER Cl^- efflux and is mutated in ALS. The ER releases Ca^{2+} via RyR and IP3R. Ca^{2+} is then taken up via SERCA. Ca^{2+} release from the ER causes accumulation of negative charges in the lumen which is counteracted by either K^+ influx or Cl^- efflux. K^+ influx is mediated by TRIC and other K^+ channels, as well as by IP3R and RyR that are known to have intrinsic K^+ permeability. Guo and colleagues show that Cl^- efflux is mediated by CLCC1, which they propose to rename ER anion channel 1 (ERAC1). CLCC1 has three transmembrane domains, and it is negatively and positively regulated by Ca^{2+} and PIP2, respectively, at the indicated residues (in blue and green, respectively). The authors also show that S263R and W267R mutations in CLCC1 (in red) contribute to ALS pathology. Created with BioRender.com.

motoneurons. These cellular phenotypes are accompanied by hind leg weakness, trunk shaking, tail flagging, abnormal gaits, and ataxia in *Clcc1(K298A)* knock-in mice.

Since ubiquitin-positive inclusions in neurons and lower motor neuron loss are hallmarks of amyotrophic lateral sclerosis (ALS), the authors perform whole-genome sequencing of a Chinese cohort and identify eight rare variants in *CLCC1* of ALS patients, including six missense and two nonsense mutations. S263R and W267R mutations are further analyzed and are found to reduce single channel activity, enhance Cl^- retention in the ER, reduce intracellular Ca^{2+} signaling, and enhance ER stress and motor neuron degeneration just as knockdown of *CLCC1* and *CLCC1(K298A)* knock-in (Fig. 1). These results support the idea that S263R and W267R are loss-of-function mutations and most likely the cause of ALS pathology in these patients.

Of note is that all three CLCC1 mutant proteins — K298A, S263R, and W267R — undergo proteasome-mediated degradation in vivo and exert a dominant negative effect on the wild-type allele. This mechanism of channelopathy has been described for other ion channels, reflects the multimeric nature of these proteins, and suggests that therapies should be aimed at repairing the mutant protein or enhancing the activity of the remaining functional channels. To conclude, data presented in this study provide strong support for the idea that CLCC1 is the long-sought ER Cl^- channel that neutralizes charge movement during Ca^{2+} release and show for the first time that mutations in this gene are linked to ALS pathology.

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ADDITIONAL INFORMATION

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