

Think Vesicular Chloride

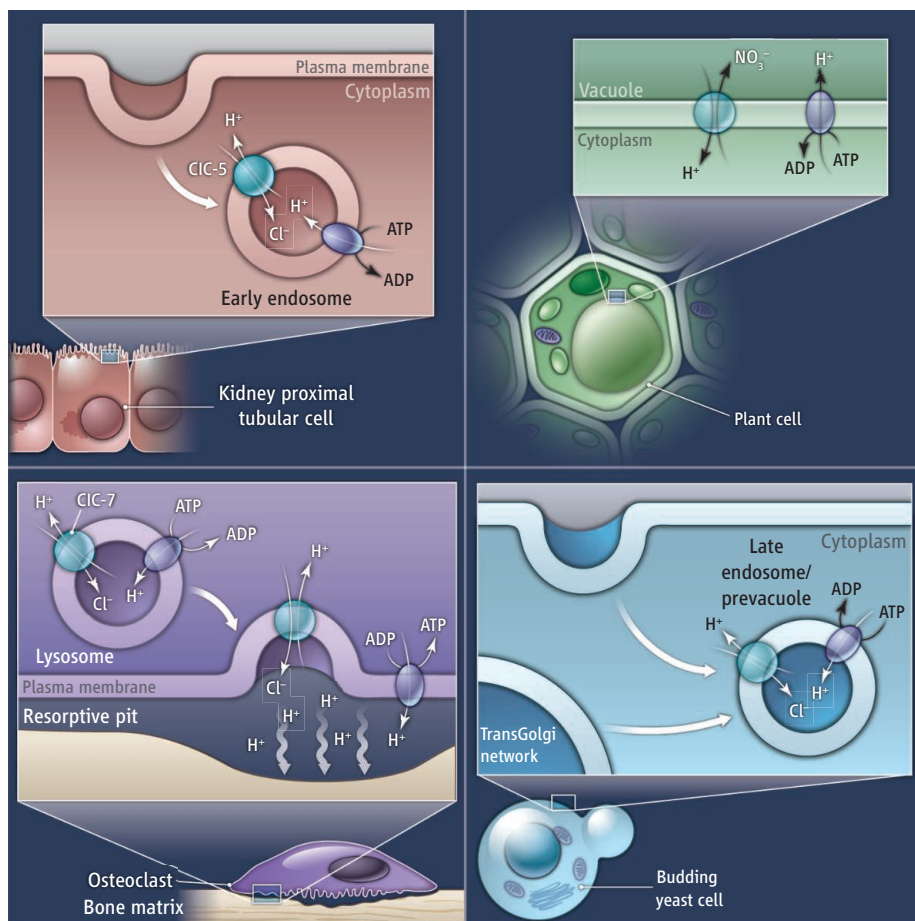
Andrew J. Smith and Blanche Schwappach

The distinction between passive and active ion movement through cellular channels and exchangers, respectively, has rarely been more relevant to understanding the physiological function of a protein than in the case of intracellular members of the chloride channel (CLC) family. Different CLC family members act either as chloride ion channels or as chloride-proton exchangers. On pages 1398 and 1401 of this issue, Novarino *et al.* (1) and Weinert *et al.* (2) demonstrate the relevance of the exchange activity in mammalian cells.

According to the prevalent hypothesis for the role of CLC proteins in endocytosis in kidney tubular cells (CLC-5) and in lysosomal biology (CLC-7), chloride moves into the organelle lumen via the CLC protein toward a positive electrical potential that a primary active proton pump has generated across the membrane. This movement of chloride dissipates the potential and enables vesicle acidification (“chloride shunt hypothesis”). Instead, Novarino *et al.* and Weinert *et al.* hypothesized that the transporter exploits the proton gradient to concentrate chloride inside lysosomes and endosomes (see the figure). Such activity was shown for the CLC-mediated accumulation of nitrate in the plant vacuole (3).

Mice genetically engineered to lack CLC-5 (*Clcn5^{-/-}*) or CLC-7 (*Clcn7^{-/-}*) are of interest because their pathology mirrors the symptoms of human disorders. The *Clcn5^{-/-}* mouse is a good model of the kidney symptoms occurring in Dent’s disease, and *Clcn7^{-/-}* mice develop osteopetrosis (abnormally shaped bones) and neuronal degeneration as observed in human patients (4). Novarino *et al.* and Weinert *et al.* compared these mice with mice engineered to express an altered form of either CLC in which a highly conserved glutamate is mutated (*Clcn5^{unc/unc}* and *Clcn7^{unc/unc}*). When this residue is altered, proton and chloride exchange becomes uncoupled, and both CLCs function as passive chloride channels (5). This approach allowed the authors to determine the relevance of the exchange activity to the pathology of either mouse. In both cases, the main symptoms

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Coupled exchange. Intracellular members of the CLC family are critical to the function of subcellular organelles in mammalian, plant, and yeast cells.

of the mice lacking either CLC protein were generally recapitulated in mice expressing the uncoupled form of either exchanger. The result thus excludes the chloride shunt hypothesis. Osteopetrosis was milder in the *Clcn7^{unc/unc}* mice than in wild-type animals, indicating that although the uncoupled transporter only functions as a chloride channel, it still serves some residual function.

The importance of coupled transport by an intracellular CLC is also observed in baker’s yeast. The single yeast CLC protein, Gef1, is required for the maturation of multicopper oxidases in a late endosomal/prevacuolar compartment. When its chloride-proton exchange activity is uncoupled (in a mutant *gef1^{unc}* strain), the mutant protein presumably acts as a passive chloride channel and the oxidases do not mature properly (6). Failure of the

Transporters that import chloride ions in exchange for the export of protons control the function of intracellular vesicles in mammalian cells.

uncoupled CLC variants to replace the wild-type proteins in mice shows their importance to endosome and lysosome function, but new issues are raised by their residual function. As these variants likely retain all properties of the wild-type proteins except for exchange activity, any differences between wild-type and *unc/unc* mice point toward roles that a chloride channel can fulfill—either by allowing passive chloride movement or by providing an interaction site for other proteins that contribute to organelle function.

It was previously proposed that CLC-5 could directly acidify early endosomes by exploiting higher concentrations of chloride inside the compartment. This gradient of chloride would drive CLC-5 to import protons—so-called secondary active acidification (5). Indeed, recent work demonstrates that CLC-5

controls endosomal acidification in a manner that is independent of a separate proton pump (7). It is unclear whether this or lack of vesicular chloride explains the endocytic defects of *Clcn5^{unc/unc}* mice. With the work by Weinert *et al.*, the field of organellar physiology has, however, taken an exciting turn. The authors report decreased chloride concentration inside *Clcn7^{unc/unc}* lysosomes but normal lysosomal pH. This implicates luminal chloride as an important parameter in endolysosomal physicochemistry. Current methodology to assess ion gradients and membrane potential across intracellular membranes *in vivo* is severely limited, and improvements in live cell imaging of these parameters will have a major impact on the field.

The findings of Weinert *et al.* support a physiological requirement for chloride along the endosomal and lysosomal pathway and raise an important question in the field: What is the function of vesicular chloride? Is it required as a cofactor of protein biogenesis as has been suggested for the yeast model (8)? Or does it control the concentration of other physiologically relevant anions by influencing their ability to move across the relevant membrane (6)? Distinct chemical reaction spaces are generally considered one major advantage of maintaining subcellular organelles. The life of organelles is not static, and the processes controlled by intracellular ion channels and exchangers may be subject to regulation, such as by metabolism (6, 9, 10).

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CHEMISTRY

Clean, Green Chiral Reactions— Just Add a Salt

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Chirality—literally handedness—refers to the mirror-image, or left-right asymmetry of objects like shoes and gloves. Like our hands, many chemical compounds (and most biomolecules) exist as enantiomers, which, relative to each other, have mirror-image arrangements of substituents around an atom (typically carbon or nitrogen). In many cases, pharmaceuticals must have a specific chirality in order to be active, but making pure chiral compounds is difficult and costly. Traditionally, chemists have relied on reagents for controlling chirality that contain heavy metals (1), the presence of which can lead to toxicity issues. A relatively new alternative is the use of nonmetal catalysts called organocatalysts. On page 1376 of this issue, Uyanik *et al.* (2) report a more “green” organocatalytic reaction that also uses an iodine reagent to close rings in a molecule during an oxidation step.

Reactions that produce chiral products—called asymmetric reactions—are evaluated in terms of their selectivity. The metric used is the “enantiomeric excess,” or ee, which is the excess of one form of the product over the other. Despite the environmental drawbacks of using metals such as osmium, chromium, or titanium, the selectivity of metal-containing catalysts approaches 98% or greater ee

for some transformations. Alternatives that have much lower ee’s will not be “green” if they are inefficient and waste reagents.

Iodine, like many metals, has the ability to become hypervalent; it can expand the number of valence electrons beyond the usual octet. Hypervalent iodine reagents have been used in many chemical reactions, including oxidations and functionalizations of multiple bonds, aromatic compounds, and a host of other substrates (3, 4). Chiral hypervalent iodine reagents have been an active area of study since the 1980’s, whereas the realm of aryl iodides as organocatalysts is a relatively new area of research (5–11). These organic compounds share a structural feature; the iodine atom doing the catalysis is attached to an aromatic ring, with the chirality incorporated onto that ring system in some way. Uyanik *et al.* have, in fact, recently explored the use of chiral aryl iodides as organocatalysts (12).

In the present study, Uyanik *et al.* took a different approach to hypervalent iodine chemistry. The oxidative power of hydrogen peroxide is exploited in that I⁻ is converted in aqueous solution to either hypoiodite (IO⁻) or iodite (IO₂⁻) (see the figure, panel A). To use these species in asymmetric organic reactions, they need to be chaperoned into organic solvents, and they need help in imparting chirality. Uyanik *et al.* chose a chiral quaternary ammonium cation to solve these problems

An ammonium iodide salt can perform selective organic oxidation reactions and replace more toxic metal catalysts.

(13). Upon binding the hypoiodite (IO⁻) or iodite (IO₂⁻), a neutral complex forms that can dissolve in both the aqueous and the organic phases. The shape and chiral nature of the quaternary ammonium cation (see the figure, panel B) is the key to imparting chirality in the product. Such molecules can interact with the organic substrate so that the oxidation reaction favors one of the handed products over the other.

Uyanik *et al.* reacted ketophenols, compounds with OH-substituted benzenes that also have a C=O bond tethered nearby, with this catalyst and hydrogen peroxide. They performed this reaction on a number of substituted phenols and obtained very good yields and selectivities regardless of the pattern of substitution. The selectivities observed were in excess of 85% ee, and greater than 90% for monosubstituted substrates (some examples were in excess of 95% ee). These selectivities represent the highest reported for a hypervalent iodine reagent-catalyzed reaction and are competitive with metal-catalyzed reactions in both yield and selectivity. Additionally, because the co-oxidant was hydrogen peroxide, the by-product from this oxidation was water.

Not only are the selectivities exciting, but the system is also much greener than metal-catalyzed reactions. The catalyst precursor is I⁻, iodide, the same anion found in iodized salt. The co-oxidant is hydrogen peroxide,

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