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A Decade of Debate: Significance of CO_2 Permeation through Membrane Channels still Controversial

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An interesting and lively discussion on the role of aquaporins and rhesus channels in the permeation of gases such as carbon dioxide across biological membranes unfolded during the Epithelial Transport Workshop, held in Strobl, Austria, from June 25–27, 2010, organized by the German Biophysical Society and the Johannes Kepler University of Linz. The classical view that such hydrophobic molecules can diffuse freely across phospholipid bilayer membranes (known as Overton's rule) is supported by measurements of Pohl and coworkers. On the other hand, experiments reported by Gros, Boron and coworkers suggest reproducible and significant channel-facilitated gas permeation.

The difficulty to resolve this long-standing issue (the literature discussion already goes back more than a decade) is a technical one. First of all, in aqueous solution, CO_2 is in equilibrium with bicarbonate, rendering it difficult to unambiguously measure absolute CO_2 concentrations. In fact, this phenomenon is exploited experimentally by the Boron group to deduce CO_2 concentration changes via a change in pH. However, this indirect assessment of CO_2 concentration is not without challenges, as the reaction between CO_2 and the bicarbonate HCO_3^- is catalyzed by the enzyme carbonic anhydrase (CA), the concentration of which therefore changes the kinetics of the reaction. Likewise, the red blood cell (RBC) CO_2 permeability estimates from the build-up of extracellular isotope labeled CO_2 after administering labeled HCO_3^- in the absence and presence of RBCs, as carried out in the Gros lab, depends not only on the CO_2 permeability of the RBC membrane, but also on the CA activity, the membrane's permeability to protons, and unstirred layer effects. In addition, the bicarbonate itself may also permeate the membrane, via anion exchanger proteins.

Nevertheless, the observed results are intriguing. Both the experiments carried out in the Gros and Boron labs observe a clear difference with and without aquaporin and rhesus channels present. Backed up by mutant and inhibitor studies, these results suggest a clear role of these channels in CO_2 permeation.

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Not presented during the workshop, but noteworthy to mention, are the experiments of Kaldenhoff and coworkers, who have proposed a role of aquaporins in CO_2 permeation in plant membranes, thereby supporting photosynthesis.

Another set of experiments, however, carried out in the Pohl group on planar membranes, suggest that rather than the membrane itself, unstirred layer effects, i.e. slow diffusion across membrane-adjacent solvent layers that are not efficiently stirred, form the main barriers to gas permeation. In this view, the membrane itself, as predicted from Overton's rule, does not pose a substantial barrier to hydrophobic molecules such as CO_2.

A substantial part of the Strobl discussion focused on the validity of artificial membranes to model the biological cellular counterparts. Roughly 50% of the RBC membrane, for instance, are occupied by proteins, and additional proteins on the intracellular side may further modulate the membrane permeability. In contrast, the membranes used in the Pohl experiments are typically composed of *E. coli* lipids enriched with sphingomyelin and cholesterol. The water permeability of these membranes closely resemble the water permeability of the oocyte membranes also studied in the Boron lab, suggesting that artificial membranes provide a valid model for biological membranes. However, it is not clear yet whether the permeation of polar water molecules requires different physicochemical mechanisms and thus may not allow one to conclude on the permeation the apolar CO_2.

Molecular dynamics simulations show that both Rhesus channels and aquaporins may be permeated by CO_2, but that the pathway across model phospholipid bilayer membranes such as POPE, POPC, or mixtures of PE, PC and PG is much more energetically favourable. High concentrations of cholesterol of 40 mol% or more increase the membrane barrier, but not to the extent that channels such as rhesus channels or aquaporins, even at high expression levels, are expected to play a major role in gas permeation. Noteworthy, whereas single-channel water permeabilities derived from aquaporin simulations are in good agreement with experimental values, the simulations predict single-channel CO_2 permeabilities for rhesus and aquaporin channels that are two orders or magnitude lower than implied by the experiments carried out in the Gros lab, which suggest large permeabilities of $\sim 10^{-12}$ cm^3 s^{-1}.

Given the persistence of the discrepancy, rather than experimental artifacts being responsible, it appears more likely that the different experiments are probing different phenomena. In live cells, due to the complex equilibria including many components and factors, it seems difficult to rule out the possibility

that rather than directly probing (channel-mediated) gas permeation, other, indirect effects may influence the interpretations. This would seem to call for well-controlled biophysical measurements, that allow the direct and unambiguous establishment of the quantitative contribution of each of the studied components to gas permeation. However, paradoxically, it cannot be excluded that a biophysical setup with a model bilayer membrane misses some of the key features of a biological membrane, therefore possibly yielding an incomplete or unrepresentative picture.

To resolve the controversy, novel and original experimental designs therefore appear mandatory. From the biophysical side, one promising direction would be to replace model membranes by ones that mimic biological tissues as closely as possible. Exact membrane compositions for different cell types are thus required to test the hypothesis if such biological membranes indeed may have a much larger intrinsic resistance against gas permeation than the model membranes studies thus far. Likewise, from a biological point of view, it would appear meaningful to design more direct ways to assess gas permeability and to reduce the number of indirect effects that may complicate the interpretation of the recorded signals.

As a next step, more relevant than the academic issue whether or not aquaporins and rhesus channels can permeate gases, is the question: do they? That is, is there a physiological role of membrane channels in gas permeation? Is there a gas phenotype? Thus far, it appears as if humans lacking aquaporin-1 or RhAG can lead relatively normal lives. However, for physiological studies on knockout mice it is still early days and interesting times lie ahead of us to resolve this exciting issue. The next epithelial transport workshop in Strobl is already planned for 2012, and it promises to be an excellent opportunity to present and discuss progress in this controversial field.

