Cell-volume regulation: **P-glycoprotein – a cautionary tale**

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P-glycoprotein turns out not to be 'VSOAC', a known channel activated by cell swelling; it does seem to influence cell-volume recovery after swelling, but the physiological importance of this effect is presently unclear.

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Animal cells swell rapidly in response to a drop in the osmolarity of their surrounding fluid. To compensate for this, the cell releases osmolytes and water follows, a process known as regulatory volume decrease. One ubiquitous and much-studied pathway for osmolyte release involves the 'volume sensitive osmolyte and anion channel' (VSOAC) [1]. An early single-channel study of the VSOAC showed that it is outwardly rectifying, has a conductance of ~50 pS in 150 mM Cl^- and, most importantly, is either almost always open or completely closed. These observations suggested that "graded increases in cell membrane Cl^- permeability [in response to cell swelling] are effected by an increase in the number of active channels rather than a graded increase in the open probability" [2].

Although neither the molecular nature of the VSOAC nor its mechanism of action have been established, considerable evidence has been marshaled to indicate that the channel may be wholly or partly composed of I_{Cln} [1], as first proposed by Paulmichl *et al.* [3]. I_{Cln} is a 235-residue protein that is endogenously expressed in most cells, but that gives rise to increased VSOAC currents when its level is increased by exogenous expression in transfected cells. Modeling suggests that a dimer of I_{Cln} may form an eight-stranded antiparallel β barrel that, like a porin molecule, spans the membrane to form a pore [3]. VSOAC's unusual channel kinetics have been attributed to the release of I_{Cln} from internal binding sites and insertion into the plasma membrane [1]. An alternative suggestion that has attracted a lot of attention is that the VSOAC is made up of P-glycoprotein. Recent results rule out this possibility, though P-glycoprotein expression does seem to influence swelling-activated conductances.

Is P-glycoprotein also VSOAC?

P-glycoprotein is a member of a large class of transmembrane transport proteins, the ATP-binding cassette (ABC) transporters, which have two distinctive nucleotide-binding domains [4]. P-glycoprotein is also known as a multidrug-resistance (mdr) protein — its expression makes cancer cells simultaneously resistant to a wide variety of chemotherapeutic drugs [5]. It has been suggested that P-glycoprotein has this effect because it exports chemotherapeutic agents out of the cells in which it is expressed. In normal mice, this promiscuous transport activity may be put to good use keeping toxins away from the brain — P-glycoprotein is abundant in cells of the blood–brain barrier, and 'knockout' mice lacking P-glycoprotein, although otherwise healthy, are highly susceptible to certain toxins which accumulate to abnormally high levels in their brains [6].

The odyssey linking VSOAC and P-glycoprotein began with a comparison of domain and sequence similarities between P-glycoprotein and the cystic fibrosis transmembrane conductance regulator (CFTR). Because the primary physiological defect in cystic fibrosis is a loss of a specific type of epithelial Cl^- conductance [7], it was suspected that the affected gene would code for a Cl^- channel. When the sequence of CFTR revealed its resemblance to ABC transporters, however, speculation began that CFTR somehow used its transport properties to function as a conductance regulator, an idea that persists to this day. When the evidence that CFTR is an intrinsic Cl^- channel became unequivocal [8,9], it was suggested that P-glycoprotein might also be a Cl^- channel, and that both molecules might have dual roles as ion channels and efflux pumps [10].

The possibility of P-glycoprotein having a channel function was tested by comparing whole-cell Cl^- currents in cells that did or did not express P-glycoprotein. It was reported that swelling-activated Cl^- currents occur only when P-glycoprotein is expressed, and it was concluded that P-glycoprotein is a swelling-activated Cl^- channel — the same channel we would now designate as VSOAC [10]. This was followed by a much more detailed study, from which it was concluded that P-glycoprotein functioned alternately as either a Cl^- channel or an efflux pump, with the two functional states being mutually exclusive [11].

These reports generated a great deal of excitement [12], but were also greeted with considerable skepticism, because almost everyone who carries out whole-cell studies with permeant anions present quickly discovers that large, VSOAC-mediated currents are ubiquitous unless pipette and bath solutions are carefully balanced to avoid cell swelling [13]. Expression of P-glycoprotein is not ubiquitous, however, and more than ten papers [14–24] have since reported the lack of a correlation

between P-glycoprotein expression and the presence of VSOAC currents. These studies used many different cell types and a wide variety of methods for manipulating P-glycoprotein and for assessing channel activity. All of them concluded that P-glycoprotein is not the VSOAC channel. It is now clear that the original interpretation was wrong: P-glycoprotein is not VSOAC.

Can the disparate results be reconciled?

In an attempt to explain both the original observations and these many failures to repeat them, we carried out a simple experiment in which the osmotic challenge to the cell was varied, while VSOAC activity was monitored either by whole-cell recordings or ^{125}I efflux [25]. This showed that cells without P-glycoprotein had large VSOAC-mediated currents, but that cells expressing P-glycoprotein were more sensitive to osmotic challenge. By carefully titrating the degree of osmotic challenge, it was possible to achieve an absolute difference between the two types of cell, as was noted in the original studies [10,11], whereas at larger dilutions the two types of cell appear equivalent.

This method has now been extended to show that expression of a mouse P-glycoprotein isoform (P-gp1a) influences only the rate of VSOAC activation, with steady-state conductance values being equivalent in whole-cell recordings [26]. Three other findings of interest have emerged from this study. Firstly, cells expressing mouse P-gp1a showed a more rapid volume recovery after swelling. Secondly, in the cell line used, the resting K^+ conductance was high, and not further increased by cell swelling, so it was not possible to determine if P-glycoprotein expression had any influence on the swelling-induced K^+-conductance increase observed previously [25,27]. And thirdly, expression of mouse P-gp1b, which is 84 % similar to mouse P-gp1a and which also confers multidrug resistance, did not influence the swelling-activated responses [26]. These findings support the authors' earlier assertions that the effects observed are specific to P-glycoprotein, and that they can have consequences for volume recovery. They also confirm the consensus finding that P-glycoprotein cannot be VSOAC.

Is P-glycoprotein a channel regulator?

How should we interpret the influence that P-glycoprotein expression has on VSOAC currents in some cells? One interpretation is that P-glycoprotein is a specific channel regulator that facilitates the rate of VSOAC activation [26]. In view of the evidence that CFTR and the sulfonylurea receptor also regulate ion channels, it has been suggested that a common regulatory mechanism might underlie all such actions [26].

A different view is that the concept of 'channel regulator' has been stretched too far, and is now used indiscriminately to describe any influence, no matter how remote, of an expressed protein on a conductance. Almost nothing is known about the steps that intervene between a hypoosmotic challenge and activation of VSOAC, but the long latency of the response indicates that the pathway might be complex. P-glycoprotein might influence any step in the pathway, and the resulting effect on VSOAC activity might be incidental.

A concrete example can best illustrate how the term 'channel regulator' can obscure understanding. When the protein Chip28 (aquaporin) was expressed at high levels in oocytes, it allowed more rapid swelling in the face of osmotic challenge. If this expression study had been carried out while measuring VSOAC currents, it might have been concluded that Chip28 is a Cl^- channel or a channel regulator. Fortunately, it was correctly determined that Chip28 forms water channels [28]. Although expression of aquaporin is expected to affect measurements of swelling-activated ion currents, its identification as a 'channel regulator' would have been misleading at best.

Does the influence of P-glycoprotein on VSOAC have physiological significance? The availability of mice lacking P-glycoprotein [6] provides the opportunity to find out, but such studies of knockout mice may be difficult, given the small and variable effects observed in cultured cells. Even with artificially high levels of P-glycoprotein, effects were small enough to be missed by many observers. Furthermore, at least one cell line selected for a high level of P-glycoprotein is less sensitive to osmotic challenge than is the unselected, parental line [27].

Considerable effort has been expended to test the hypothesis that P-glycoprotein encodes VSOAC. That hypothesis was disproved, and the evidence that P-glycoprotein expression is correlated with an altered response of VSOAC, at least in some cell lines, is at present a laboratory curiosity with no link to physiology. There is as yet no evidence that P-glycoprotein is a specific, direct regulator of VSOAC. More research may yet establish a relationship of deep and general importance, but such research is unlikely to be informative until the molecular basis of VSOAC has been definitively established, and until more is known about how cell volume changes are signaled to VSOAC and other cellular elements involved in cell volume regulation [1].

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