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BACTERIAL AND PLANT ANTIPORTERS

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Articles in this chapter emphasize the phenomenon of ion exchange across biomembranes. This topic has been of interest at least since 1947, when Hans Ussing articulated his model of 'exchange diffusion' to account for an unexpected elevation in the rate of $^{22}Na^+$ efflux from skeletal muscle (Ussing, 1947). Ussing had tried to measure net Na^+ movement by following tracer flux, but found that this approach greatly overestimated the true value. To explain this overestimate, Ussing postulated that isotopic flux reflected only isotopic exchange rather than net movement of mass, and he proposed the following model. Perhaps a negatively charged carboxyl group on some membrane element accepts the positively charged $^{22}Na^+$ so that the neutral complex can move through the membrane (mechanism unspecified) without interference from the electric field? If so, at the opposite surface, tracer could be discharged, replaced by (non-radioactive) $^{23}Na^+$, and the neutral complex would return to the original membrane surface, and at that point $^{23}Na^+$ would be released to let the cycle begin again. Clearly, by following the movement of $^{22}Na^+$, one might considerably overestimate the true net movement of Na^+ itself.

Note that Ussing's conceptual analysis of exchange is the same as that offered by Widdas (1952) in the latter's presentation of the more general idea of a membrane carrier. Moreover, the idea that ion pairs might constitute essential elements in the movement of charged compounds is as useful today as it was in 1947. Indeed, we need only modify Ussing's proposal in minor ways to arrive at a view of ion exchange pertinent to the topics treated here. First, we would specify that the catalyst for transport is a specific kind of membrane protein – for example, one having 10–12 transmembrane helices, this being the most prevalent structural element found among the various porters now known. Second, we would note that the physiologically relevant mode of exchange must be a heterologous, not a homologous, event – that is, that Na^+ might exchange with H^+ (as discussed in this chapter) or Ca^{2+} or K^+ or some other cation; or that one anion might exchange with another anion – inorganic phosphate (P_i) with glucose 6-phosphate (discussed here), ADP with ATP, or perhaps Cl^- with HCO_3^-. I note that, although not unimaginable, there is no good example of an exchange reaction in which the carrier mixes passengers of opposite charge; this deficiency points to an instructive role for the pairing of charge(s) between porter and passenger, as first suggested by Ussing.

Subsequent to Ussing's work, cation exchange systems continued to be of theoretical interest. In particular, they played an important role in the chemiosmotic hypothesis of Peter Mitchell (Mitchell, 1961). In that case, such 'antiporters' (as we now call them) were considered to be essential if mitochondria were to express a membrane potential,

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negative inside the organelle, for over the long term cytosolic Na^+ and K^+ would inevitably accumulate, driven inwards by the electrical potential and, unless some mechanism were available for cation extrusion, swelling and lysis would be just as inevitable. It was to avoid such an osmotic crisis that Mitchell proposed a series of cation exchange reactions to enable the net extrusion of, say, Na^+ or K^+, in exchange for protons.

The reviews by Padan and Schuldiner (1994) and by Krulwich *et al.* (1994) carry forward from these early ideas and document that the experimental study of cation exchange is alive and well, and that microbial systems present especially sophisticated examples for study. We now see many roles for cation exchange, at least three of which are discussed here, with specific reference to the antiport of Na^+ and H^+; there is such high value placed on this single reaction that it is nearly universal in its distribution among microorganisms. First, entry and accumulation of external Na^+ presents a significant physiological load inasmuch as such events as protein synthesis proceed more rapidly and with more fidelity in the presence of K^+. Second, and perhaps most important, Na^+/H^+ antiport (or any other such proton-linked exchange) serves as the intermediate that connects ionic circuits of different selectivity. For example, by extruding Na^+ that enters during Na^+-coupled solute symport, the Na^+/H^+ antiporter ensures a stable integration of two ionic circuits – the one selective for H^+, the other for Na^+ (see Fig. 1 in Maloney *et al.* 1994). (Note that this is as true in bacteria, where H^+ movements are primary, as it is in animal cells, where Na^+ circulations dominate.) A third role of Na^+/H^+ antiport is in the interplay between H^+ and Na^+ homeostasis. Although this function is least well-defined, it appears that in both *Escherichia coli* and *Bacillus subtilis* an electrogenic Na^+/nH^+ antiporter allows the efflux of Na^+ to play a role in cytoplasmic acidification when stressful alkaline conditions are faced. In the same way, an electrogenic K^+/nH^+ antiporter allows K^+ outflow to generate extracellular alkalinity in the gut of lepidopteran insects (Lepier *et al.* 1994).

I should also note that the capacity to manipulate cation exchange systems in bacteria – specifically, the ability to delete them from the *E. coli* chromosome – offers an unprecedented experimental opportunity that is fully appreciated by workers in the field. Such defenseless cells are Na^+-sensitive and form an ideal selective host for identifying other mechanisms which extrude Na^+ if over-expressed, but which do not normally do so for reasons of low velocity, poor affinity or lack of induction. This tactic has already paid off in the cloning of a Ca^{2+}/H^+ antiporter, and we anticipate more cases in the future. An especially good example of how this approach leads in unexpected directions is the discovery that a tetracycline export system in *B. subtilis* may have been derived from a Na^+/H^+ exchanger (Krulwich *et al.* 1994).

Anion exchange systems are also represented in bacterial systems and here, too, there is an important historical element. The very first microbial transport system to be described – the phosphate self-exchange system of *Micrococcus pyogene*s (now *Staphylococcus aureus*) (Mitchell, 1953) – belongs to the 'P_i-linked' family of anion exchange discussed by Maloney *et al.* (1994). Contemporary studies of this family focus on molecular biology, and this emphasis promises important insights into the general nature of membrane transporters. One may now hope to describe directly the substrate translocation pathway by monitoring, within the transport protein itself, the volume of

distribution of a universally reactive probe whose size and charge resemble those of the true substrate. In this case, note that universal reactivity is achieved, not by chemistry, but by site-directed mutagenesis, as cysteine residues are placed one at a time at various positions in the carrier. The approach has already identified one residue in the translocation pathway and experimental criteria are available to support a more extended effort (see Maloney *et al.* 1994). It is certain that this experimental design will be heavily exploited in the coming decade, for work with all kinds of transport proteins; in fact, several ion channels have already been the target of such work (see Akabas *et al.* 1994).

The study of anion exchange is yielding an increasingly detailed molecular picture of transport and transporters. It is gratifying, therefore, that such work is also contributing to a wider appreciation of physiology. We now know that, in bacteria at least, anion exchange participates in an unusual multi-component organizational scheme, one incorporating both vectorial (transport) and scalar (metabolic) events, that comprises a proton pump (Maloney *et al.* 1994). This new perspective on ionic circuits represents, as does the probable origin of tetracycline-resistance (see above), a lesson in how a traditional field of study might replenish itself.

The final topic treated in this chapter concerns ion-motive events in plants and in plant model systems. Widmar Tanner summarizes an elegant series of experiments on the proton-coupled hexose transporter in lower and higher plants (Caspari *et al.* 1994). The phenomenology of symport is discussed elsewhere in this volume (Kaback *et al.* 1994; Wright *et al.* 1994), so I would direct attention to other areas of note in Tanner's review. Of special interest is the fact that the plant hexose transporters function well in yeast. Yeast cells, then, can serve the plant biologist as *Xenopus* oocytes serve the mammalian biologist – as a powerful system for cloning and expression of relevant proteins. But yeast cells are themselves amenable to genetic analysis, whereas *Xenopus* is not. The use of yeast should therefore be of enormous value to the general field of plant membrane biology. One envisions that the continuing generation of null mutants in yeast will support the expression cloning of their plant equivalents and that in the near future we will know nearly as much about membrane cell biology and biochemistry in plants as we do in yeast (and possibly more than we know in mammalian systems). Certainly, the experiments described by Tanner (Caspari *et al.* 1994) show how this expectation might be fulfilled. If only there were an equally versatile system for expression cloning of mammalian membrane transporters. Unfortunately, for reasons still unclear, it has been difficult (if not impossible) to use yeast or bacteria as hosts for mammalian membrane proteins. We look forward to some future PORTER meeting to hear how this issue has been solved.

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