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Review

On proton-coupled information transfer along the surface of biological membranes and the mode of action of certain colicins

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1. INTRODUCTION

The colicins are a heterogeneous group of proteinaceous bactericidal agents produced by a variety of bacteria and active against many strains of *Escherichia coli*; many other bacteriocins active against other bacterial species, and exhibiting broadly similar properties to some of the colicins, have also been described (for review see [1–3]). As Plate has recently pointed out in a short review [4], certain of the colicins, especially those of the E1, K and Ia types, which are known to disrupt membrane energy transduction processes in sensitive bacterial strains, may prove extremely useful as probes of the nature of membrane energy transduction processes themselves.

The purpose of the present article is threefold. Primarily, to develop the idea that energy-transducing membrane systems normally contain a number of proteinaceous components whose role is to act co-operatively as conformationally switchable proton conductors, permitting fast, controlled *lateral* proton transfer along the surface of such energy-transducing membranes, and acting as the major energetic links between the various protonmotive sources and proton-accepting sinks embedded in such membranes. Secondly, to draw together evidence that the elements of such a "protoneural" network are themselves the prime target of the membrane-active colicins, and, thirdly, to point out that the recognition that such a network is an important feature of proton-coupled energy-transducing systems in vivo both provides a ready explanation for a variety of data apparently at odds with most presently accepted schemes of protonmotive energy transduction and renders intelligible a number of experimentally observable features of such systems for which a unifying view has not previously been offered. As will be clear from the following, we make no claim to the originality of many of the ideas presented here; we do believe, however, that our attempt to meld the conclusions drawn from a variety of experimental approaches into a unifying model will be helpful to those concerned with membrane energy transduction processes, their physiological roles and their molecular mechanisms. We begin with an outline summary of current ideas concerning the nature of energy transduction processes catalysed by membrane-located proteins.

2. ENERGY TRANSDUCTION BY BACTERIAL MEMBRANES

Current thinking on the question of free energy transduction (and thus information transfer) by bacterial membrane systems is dominated by the

realisation that "energised" protons can act as mobile coupling intermediaries between different processes catalysed by membrane-located enzymes. Within this framework two main types of view may be recognised. According to the chemiosmotic approach, pioneered by Mitchell (e.g. [5–10]) and reviewed many times in relation to microbial growth and metabolism by others, for instance by Harold [11–13], Hamilton [14,15], Garland [16], Haddock and Jones [17] and Konings and Veldkamp [18], the proton flux between protonmotive sources and sinks is carried entirely in the bulk aqueous phases separated by the coupling membrane, which itself serves merely as an insulating osmotic diffusion barrier. In this view there is no decrease in electrochemical protonic potential between the surfaces of the coupling membrane and the bulk of the aqueous phases to which they are adjacent [6].

In an alternative view, initially proposed by Williams [19–22], and now espoused by an increasing number of other workers (for a recent review see [23]), under coupled conditions in vivo the "energised" coupling protons do not themselves quantitatively enter the bulk aqueous phases but remain membrane-associated (i.e. inside the electrical double layer at the membrane/solution interfaces). In this view, the undoubted protonmotivated passage of other ions and solutes between the bulk phases is not, under normal circumstances, accompanied by proton movements between these phases, and the membrane itself is viewed as the site of free energy storage (for an excellent definition of this term see [24]). An important distinction between this "energised membrane" view and the strictly chemiosmotic view is that in the former the possibility of localised lateral channelling of the proton currents, mediated by chains of hydrogen-bonded acid-base groups (including proteolipids and structured water), is emphasized, whilst in the latter (chemiosmotic) view no such possibility exists. Thus the view that it is the membrane which becomes "energised" as a result of protonmotive activity, and not the bulk aqueous phases which it separates, implies the existence of what amount to "proton wires" (cf. [25,26]; an inappropriate term, this, since it implies a purely passive role) for the rapid conduction of energised protons along the surfaces of the coupling membrane. It is implicitly assumed that such a conduction will be controlled by cooperative protonmotivated conformational changes of membrane-located proteins. A diagrammatic representation of these two types of view is given in Fig. 1.

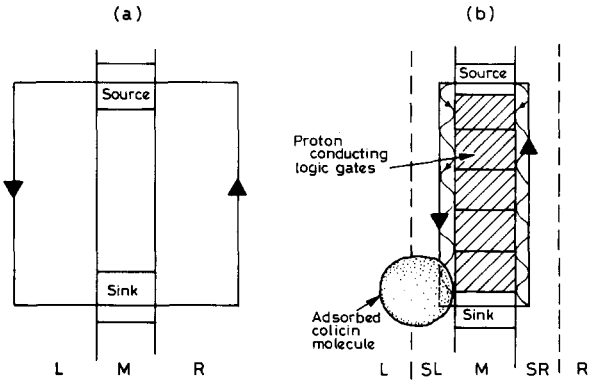

Fig. 1. Diagrammatic representation of two (purely 2-dimensional) models for the proton current pathway of membrane energy coupling. In the more classical chemiosmotic representation (a) the membrane is viewed as a passive diffusion barrier (M phase) separating two aqueous phases (L and R) wherein the proton current is carried. In an alternative view (b), however, the proton current is carried within the membrane/solution interphases (SL and SR), aided by conformationally switchable protonic "logic gates", constituting a protoneural network. A single adsorbed colicin molecule, by binding to an element of such a network, could cause a cooperative conformational transition in this network, thereby inhibiting energy coupling. Arrows represent the flow of protonic current.

We shall here seek to show that, as intimated elsewhere [23], what is currently known of the primary mode of action of membrane-active colicins is explicable only within the framework provided by the "energised membrane" view of membrane energy transduction. We begin with an analysis of the broadly chemiosmotic type of interpretation which has been used to explain certain observed physiological effects of membrane-active colicins, before turning to the numerous features of their activity which suggest that their prime mode of action actually lies in their ability to disrupt the normal functioning of an arcane but comprehensive membrane-located protoneural network.

3. PHYSIOLOGICAL EFFECTS OF MEMBRANE-ACTIVE COLICINS

As recently reviewed [1–3], it is now widely understood that the primary site of bactericidal action of colicins of the E1, K, and Ia types (which we shall refer to as membrane-active colicins), as well as of other membrane-active bacteriocins such as staphylococcin 1580 characterised by Vogels and co-workers (e.g. [27,28]) and butyricin 7423 studied in this laboratory [29–31], is at the energy-transducing cytoplasmic membrane of sensitive organisms. (We make no attempt to discuss the mechanisms by which such bacteriocins penetrate to this membrane.) Thus the membrane-active colicins were found to inhibit certain respiration-linked active transport systems and cellular motility [32], to lower cellular ATP levels [33,34], to cause a rapid efflux of intracellular K^+ [35–39] and to inhibit the energy-linked transhydrogenase reaction [40] in sensitive cells. However, electron transport activity in treated cells continued unabated, and inhibition of ATP synthesis via the membrane-bound H^+-ATPase was not apparently implicated in the bactericidal action of the bacteriocin since *uncA* mutants of *E. coli* (which lack this activity [41]) were also sensitive to colicin (B. Rolfe, cited in [1], cf. [42]). Observations such as this led logically to the view that membrane-active colicins dissipate the "energised state" of the membrane, and that indeed if we knew exactly what constituted this "energised state" we might comprehend the mechanism of action of such bacteriocins.

From a chemiosmotic standpoint it appeared reasonable to suppose that these colicins might be classical protonophores, in that the addition of a protonophorous uncoupler would be expected to inhibit all protonmotivated bioenergetic processes in aerobic organisms. However, it was found that colicin E1 exhibited no protonophorous activity and that its action could not be mimicked by known protonophores [38,43,44]. It was therefore supposed that membrane-active colicins might act to discharge the bulk-phase protonmotivated transmembrane electrical potential difference, perhaps by acting as K^+-ionophores, although it was recognised [1] that this activity alone would neither cause a classical uncoupling action nor adequately mimic the physiological effects of these colicins. However, this broadly chemiosmotic interpretation was apparently greatly strengthened by the observation that membrane-active colicins do indeed decrease the steady-state transmembrane electrical potential measured using membrane-permeable phosphonium salts (a method believed to measure only the bulk-phase $\Delta\psi$ [45]) both in intact cells [3,46,47] and in cytoplasmic membrane vesicles [3,47,48]. Parenthetically, we feel bound to point out that these observations were made using either very large concentrations of triphenylmethyl phosphonium salt [47], or with the addition of tetraphenylborate [46] or following the application of a freeze-thaw cycle [3,48], and may thus not be quantitatively representative of the situation in vivo. It was also demonstrated that the addition of colicin K to phospholipid bilayer membranes resulted in the formation therein of a rather unselective, voltage-dependent, gated ion-permeable channel [49]. These latter workers drew together a number of previous observations, including the rates of colicin K-induced K^+ efflux observed by Wendt [39], and suggested that the pathophysiology of colicin K action could be adequately explained by the view that it acts merely to form a poorly-selective ion channel across the bacterial cytoplasmic membrane. Yet, despite this poor selectivity for larger anions and cations, it was necessary to concede that such a channel would be proton-impermeable, since no colicin-induced decrease in ΔpH is observed [3,46,47,50]. In view of the evidence just described, therefore, it might be thought that the mode of bactericidal action of membrane-active colicins, at the minimal concentrations that are lethal, had been established, and was, by implication, fully consistent with a strictly chemiosmotic interpretation of bacterial membrane energy transduction. We believe this conclusion to be in error, however, and we therefore devote the next section to an analysis of certain inadequacies in its basis.

4. SINGLE-HIT KILLING BY COLICINS

One of the most remarkable features of the action of membrane-active colicins is that they

appear to exhibit single-hit killing (see e.g. [1,2,51,52]); in other words, a single adsorbed colicin molecule has a finite probability of killing an entire cell. This phenomenon may be explained, in principle, on the basis of any of at least three mechanisms: (i) reproduction of the lethal element, (ii) enzymatic mediation of cytotoxicity, or (iii) propagation by means of cooperative conformational changes in information-carrying macromolecules. According to the "gated aqueous pore" theory of membrane-active colicin action [49], single-hit killing would require that the single channel ionophoric activity of colicins in vivo be sufficient to account for the rather rapid loss both of intracellular K^+ and of the steady state membrane potential. In the model outlined by Schein et al. [49], it is suggested that single colicin K molecules form (rather poorly) cation-selective pores in the bacterial cytoplasmic membrane, which are gated so that they are open all the time when the potential across the membrane exceeds approx. 50 mV, and are closed all the time if the potential is less than approx. 10 mV. The direction of field-induced current flow is not altogether clear from the paper of Schein et al. [49], but since the putative colicin pore is cation-selective we may take it that at adequately high voltages the current flows towards the negative compartment of their potentiostatted black lipid membrane (BLM) system. It should be noted that the supposed channel is completely closed when the potential is made positive in the compartment opposite that to which the colicin is added. It may be mentioned here that the amounts of colicin used in these BLM experiments were such that the amount of colicin (0.7 μg) in one of the aqueous compartments which the BLM (area 0.01 mm^2) separated is equivalent, on the basis of membrane surface area (assuming that 1 mg bacterial protein is enclosed by a total cytoplasmic membrane area of approx. 400 cm^2 [13]), to a concentration far in excess of that required for bactericidal activity). Schein et al. [49] then calculated from the rate of K^+ efflux from colicin K-treated cells of *E. coli* observed by Wendt [39] that the effective rate of passage through a single colicin channel (10^{-18} mol/s) is comparable to that observed by them as the conductance of a single channel under "fully open" conditions in their BLM system. This analysis was based, inter alia, upon the assumption that the bulk-phase transmembrane potential across the bacterial cytoplasmic membrane remained at 58 mV (negative inside), although the basis for this supposition remains completely unclear; the statement "given the selectivity of the colicin channel" appears enigmatically in the analysis of Schein et al. at this point, as though in support of this assumption. However, if the observed *dissipation* of the bulk phase electrical potential (also negative inside) across the bacterial membrane that is elicited by membrane active colicins is to be catalysed by a directly ionophoric action, in spite of any attempts that the bacterial respiratory chain might be expected [53,54] to make to maintain its $\Delta\psi$, then a net *influx* of cations (or efflux of anions) must accompany colicin action. Thus, any attempt to correlate the single-channel conductance of the putative colicin pores both with observed rates of loss of intracellular K^+ and with the dissipation of the bulk-phase $\Delta\psi$ must of necessity fail, since the requisite ion fluxes are in opposite directions. It may be mentioned that the proton current in growing *E. coli* is approx. 10 $\mu A/cm^2$ [13], whilst the single-channel conductance of the colicin pore is less than 1 $\mu A/cm^2$ [49]. Therefore, given the claimed ion selectivity of the colicin channel, we conclude that the purely ionophoric mechanism suggested [49], though superficially attractive, is in fact consistent neither with single-hit killing, nor with the rapid dissipation of the bulk-phase $\Delta\psi$, and certainly not with the observed rates of K^+ efflux (loc. cit.). Further, this type of model, ascribing a completely passive role to the protein components of the membrane (and indeed to the membrane generally), can offer no convincing explanation for the following two extremely important features of the action of such colicins: (i) very large changes in membrane structure as revealed by fluorescent probe techniques, and (ii) the existence of a number of colicin-insensitive mutant strains which have been shown beyond reasonable doubt to possess certain modified cytoplasmic membrane proteins (see later).

Thus, the foregoing, strictly (though internally inconsistent) chemiosmotically based analysis, suggesting a directly ionophoric type of action for

membrane-active colicins and a completely passive role for the cytoplasmic membrane itself, contrasts markedly with the following alternative type of analysis which has been adopted by certain other groups of workers and which more nearly approaches the "energised membrane" type of view of energy transduction outlined earlier.

Though the interpretation which we shall seek to put on the findings described in the next section is in broad agreement with the view espoused by, for example, Cramer and coworkers, Hong and coworkers and by Plate, we have chosen to lay special emphasis upon the view that the changes in membrane structure elicited by colicins E1 and K reflect their disruption of a protoneural network, rather than their inhibition of purely proton-independent, energy-transducing conformational changes.

5. THE EFFECT OF COLICINS E1 AND K ON THE RESPONSE OF MEMBRANE-ASSOCIATED FLUORESCENT PROBE MOLECULES

As an alternative approach, Cramer and coworkers [55–59], Brewer [50,60] and others [61,62] have investigated the effects of membrane-active colicins on the conformational state of the cell envelope and cytoplasmic membrane of *E. coli* as inferred from the fluorescence of bound "probe" molecules. In contrast to the "redistribution" type of probe [63], a category into which the non-fluorescent membrane-permeable phosphonium salts also fall, the types of probe used by these workers do not cross the bacterial cytoplasmic membrane when they undergo changes in fluorescence in response to changes in membrane structure. Thus these probes, although undoubtedly capable in some cases of responding to a transmembrane potential, truly reflect in some fashion the structural state of the membrane itself.

8-Anilinonaphthalene-1-sulphonate (ANS), used for instance by Cramer and Phillips [55], is well known to respond to (presumably) proton-motivated "energisation" of membranes elicited by respiration or ATP hydrolysis in an uncoupler-sensitive manner (e.g. [23,64]). It is now understood that ANS responds to changes in the potential and physical structure of the Stern layer adjacent to biological membranes [65], and, although the nature of the probe responses is still not entirely understood, it is widely accepted that such responses reflect conformational changes in the proteolipid membrane organisation (in the case of intact cells in the organisation of both cytoplasmic membrane and cell envelope). The same is true for the neutral probe *N*-phenyl naphthylamine (NPN) (which does not respond to a membrane potential and which also binds to the *E. coli* cell envelope), also studied by Cramer and coworkers [56–58] and by Tecoma and Wu [62]. In all cases, membrane-active colicins were able to inhibit the "energisation"-dependent fluorescence changes of these probes. An especially important feature of the work with ANS [55] was the observation, consistent with single-hit killing, that the colicin-induced ANS response was an all-or-none phenomenon, occurring at a multiplicity of colicin molecules of <2 per cell. This observation may thus be viewed as consistent with an earlier model of Changeux and coworkers [67,68] espoused by Wendt [39] that a gross, energy-dependent, cooperative conformational transition is a general property of coupling membranes, and that by inhibiting such a cooperative transition the single-hit killing action of colicins could be readily accounted for.

Brewer [50,60] studied the effects of colicin K on the state of membrane energisation in sensitive cells using the fluorescent probes chlorotetracycline [50] and 3,3′-dihexyloxacarbocyanine [60], and noted that the apparent colicin-induced membrane depolarisation could not be accounted for by a cationophoric activity of the colicin.

Thus, the studies reviewed in this section indicate that colicins do indeed reverse "energisation"-dependent structural changes in coupling membranes, and that a model of the type outlined by Changeux et al. [66,67], postulating highly cooperative ligand-induced conformational changes in biological membranes, can indeed neatly account for the single-hit killing by colicins and the all-or-none response of these probes. One such ligand (mentioned by Changeux et al. [67]) is of course the proton, most pertinently to our analysis the

energised proton, and to harmonise the types of model of membrane energy transduction outlined above we would suggest that all the observations reviewed thus far are best accounted for by a model in which energised protons are shuttled across membrane surfaces, the fields that they themselves establish thereby influencing the conformational state and proton conductivity not only of the eventual proton sinks but also of the proto-neural network. In this regard it is by no means unexpected that these colicins initially react only with an energised cytoplasmic membrane (see [2,3,68]).

That the "bulk-phase proton conduction" view, ascribing no possible role to membrane-associated lateral proton conduction, has perhaps been so persuasive in the past is due in part to the well-known observation, made under special conditions, of oxygen-induced proton efflux into the bulk aqueous phase external to anaerobic suspensions of aerobic respiratory bacteria [69]. We therefore devote some space to an analysis of this type of observation.

6. OXYGEN-INDUCED PROTON EFFLUX BY AEROBIC RESPIRATORY BACTERIA

Following the protocol established by Mitchell and Moyle [70] for mitochondria, Scholes and Mitchell [69] demonstrated that pulses of oxygen added to anaerobic suspensions of *Micrococcus* (now *Paracoccus*) *denitrificans* elicited the vectorial ejection of protons into the bulk aqueous phase external to the bacteria, where they could be detected with a glass electrode. In the absence of added valinomycin (an electrogenic K^+-ionophore) or the thiocyanate ion (a membrane-permeable chaotropic anion), both the rate and extent of oxygen-induced proton efflux were small, the addition of either of these compounds increasing markedly both the rate and extent of the oxygen-induced proton efflux. The role of these compounds was suggested [69] to lie in catalysing the dissipation of a bulk-phase $\Delta\psi$ built up as a result of electrically uncompensated transmembrane proton translocation. However, studies with *E. coli* by Gould and Cramer [59,71] and more recently by Gould [72] (cf. [73] for similar data in mitochondria) have cast extremely serious doubt upon the purely ionophoric role of these compounds during oxygen-pulse experiments, and Kell [23] has calculated that, for SCN^- at least, an additional non-electrogenic role must be sought for these compounds in increasing the apparent rate and extent of oxygen-induced proton efflux. Gould and Cramer [59] and Gould [72] concluded that their results were best explained by a model in which, in the absence of valinomycin, thiocyanate or colicin E1, vectorially translocated protons, accumulate in a region of the cell which is not in rapid equilibrium with the external phase, a region which the model of Kell [23] suggests, is constituted by chains of hydrogen-bonded molecules at the membrane/solution interface.

Importantly, although colicin E1, even at a multiplicity of one [74], can mimic valinomycin and thiocyanate in stimulating proton ejection into the bulk aqueous phase [59], it was concluded that a purely ionophoric activity of the colicin was indeed insufficient to account for this stimulation, as variations in the ionic composition of the suspending medium had little effect upon the observations. Further, the fact that the onset of membrane "energisation" monitored by NPN was at least ten-fold more rapid than the observable oxygen-induced proton efflux [72] provided strong evidence that normally most of the protons translocated across the membrane were used to induce protonmotivated conformational changes in membrane components before finally flowing to proton sinks such as the H^+-ATPase via a non-bulk-phase pathway.

Such observations, as well as a great many others reviewed in more general terms elsewhere [23], lead naturally and unequivocally to a model in which the majority of the functional proton current of energised coupling membranes is carried along a series of hydrogen bonded networks at the membrane/solution interfaces, and that treatment with certain membrane-active compounds can disrupt the normal flow of this proton current. With the realisation that the integrated functioning of these proton-carrying entities may be a cooperative process, it is logical to suggest that the prime site of action of membrane-active

colicins is the network of proton conductors itself, a view very greatly strengthened by the studies of Hong, Lieberman, Plate and coworkers reviewed in the next section.

7. ON THE NATURE OF *ecf* AND *eup* MUTANTS OF *E. coli*

The study of mutant strains of bacteria constitutes a powerful method for the analysis of bacterial energy coupling [41,75–78]. Several mutant strains of *E. coli* relatively insensitive to certain membrane-active colicins have been isolated (we exclude from our discussion all mutants which have a lesion either in the specific adsorption of the colicin to receptor sites in the outer membrane or in its transmission thereafter to the site of action in the cytoplasmic membrane). Those isolated by Hong and Lieberman and coworkers and by Plate are of especial interest.

Lieberman and Hong [79] isolated a (neomycin-resistant) mutant strain of *E. coli* that was pleiotropically defective in the coupling of respiration-derived energy to active substrate transport. It was subsequently noted [80] that other properties of this temperature-sensitive *ecf* mutant at the non-permissive temperature (including a fall in intracellular ATP, leakage of metabolites and the cessation of macromolecular synthesis) closely resembled reactions elicited by the treatment of sensitive cells with membrane-active colicins, and it was suggested that the protein coded for by the *ecf* gene might indeed be the target of these colicins. Later work [81] showed that this mutant was neither able to synthesize ATP at the non-permissive temperature nor to generate a bulk-phase $\Delta\psi$. It was concluded that, since both respiration and ATP hydrolase activity were normal at the non-permissive temperature, the defect attributable to the *ecf* mutation, which maps [81] at minute 64 on the revised *E. coli* linkage map [82], lay in an inability of the mutant to couple energy released at the membrane level to active transport or to ATP synthesis. Since the mutant phenotype was expressed in cytoplasmic membrane vesicles [80], it was further concluded that the product of the *ecf* gene was resident in the cytoplasmic membrane.

Hong [83] isolated another *ecf* mutant (designated strain JSH 270) which was similar to the previous mutant in that it was unable to couple respiration-derived energy to active transport, but differed in that it was still capable of maintaining a bulk-phase $\Delta\psi$. Hong [83] concluded that a model of energy coupling involving a conformational coupling factor distinct from the protonmotive electron transport and ATP synthase complexes, as suggested explicitly by Ji [84], best explained his data, a conclusion with which we fully concur, save for our additional emphasis that this conformational energy is transferred spatially by means of interfacial protons.

Hong et al. [85] next specifically isolated a colicin K-insensitive *ecf* mutant of *E. coli*, and inferred from its properties and those of revertant strains that the basis for colicin K-insensitivity indeed lay in the *ecf* gene product itself. This particular mutant was still sensitive to colicin E1.

Finally, Tomochika and Hong [86] isolated a third type of *ecf* mutant (*ecf*-17^{ts}). Like the other *ecf* mutants this mutant was unable to couple metabolic energy to active transport; however, the expression of this mutation required actual growth of the organism at the non-permissive temperature, and was accompanied by an increased permeability of the cytoplasmic membrane to protons and to nucleotides. It was concluded that the mutant gene product was inserted into the cytoplasmic membrane in such a way at the non-permissive temperature as to cause a massive general structural derangement of this membrane.

Plate [87] isolated a different (neomycin-resistant) colicin K-insensitive mutant of *E. coli*, which exhibited a broadly similar phenotype to that of Hong's colicin K-insensitive *ecf* mutants in that coupling of metabolic energy to active transport was blocked at the non-permissive temperature. This mutant, designated *eup* [4], most resembled Hong's JSH 270 mutant [83] in that it was capable of maintaining a bulk-phase $\Delta\psi$ at the non-permissive temperature [4]. It is clearly not an *ecf* mutant, however, since it maps at minute 86.5 [4] on the revised *E. coli* linkage map. Thorbjarnardottir and coworkers [88] have isolated a similar mutant to that of Plate [87] on the basis of resistance to a broad spectrum of aminoglycoside

antibiotics; its mutant allele also mapped around minute 87, and they have (somewhat confusingly) termed it an *ecfB* mutant. It is, however, apparent that the *eup* gene product has a very similar function to that of the *ecf* gene product. We therefore conclude that these gene products (and doubtless others) normally function as separate building blocks in a comprehensive protoneural network, located in the cytoplasmic membrane of *E. coli*, functioning to channel energised protons between their membrane-located sources and sinks, and forming the prime target of membrane-active colicins. Such a conclusion, which we ourselves find persuasive, implies that the bulk-phase transmembrane potential in bacteria, which in any event in *E. coli* is demonstrably largely a Na^+/K^+ diffusion potential [89], is not *directly* protonmotivated but results from secondary ion movements in response to the primary proton translocation between one membrane surface and the other [23]. The role of the bulk-phase pH gradient, therefore, lies not directly in energy coupling but rather in the regulation of intracellular pH [13,90–93].

8. SOME CONSEQUENCES OF THE VIEW OUTLINED ABOVE

Whilst we feel that the acceptance of this type of model of energy coupling greatly assists the understanding of much experimental data on the mechanism of the primary action of membrane-active colicins (lethality may well be due to various repercussive effects such as the loss of intracellular ions), we feel that it possesses certain additional advantages over most other available models. First, as reviewed elsewhere [23], life in highly alkaline environments and under other conditions in which the bulk-phase protonmotive force is far too small to account for the intracellular phosphorylation potential (cf. [94]), becomes comprehensible. Secondly, the question of how cells distribute the proton gradients generated by electron transport between the various energy-requiring processes such as active transport, ATP synthesis by individual ATPase enzymes (e.g. [95] and references therein) and flagellar rotation, becomes accessible. Thirdly, the mode of inhibition of energy coupling by membrane-impermeant lipophilic substances (e.g [96,97] and references therein; also cf. [98]), may be simply viewed as an inhibition of proton conduction along the protoneural networks. Fourthly, the manner in which such a variety of energy-uncoupled mutants may be isolated (e.g. [41,88,99–101]) by selection for resistance to both charged and uncharged aminoglycoside antibiotics, suggests that the conformational state of at least part of the protoneural network modulates the rate at which such antibiotics are taken up by bacterial cells. Fifthly, it allows a ready harmonisation of the widely accepted general theory of protonic coupling with the perhaps controversial view [102] that the bulk-phase mitochondrial membrane potential during ATP synthesis is in fact energetically insignificant.

We resist the temptation to deal with further aspects of this type of model, although we are, of course, alive to the possibility, as indeed stressed explicitly (though in a strictly chemiosmotic framework) by Harold [13] and by Mitchell [10,103,104], that vectorial proton conduction may simply account for a variety of observations concerning the mode of signal transmission in a plethora of other bioelectric phenomena, of which we consider morphogenesis, circadian rhythms and even acupuncture to be the most significant [e.g. 105–107]. A discussion of the necessary molecular mechanisms by which such a protoneural network can act to catalyse proton and information transfer by surface conduction over long distances, without permitting (under normal circumstances) the equilibration of interfacial protons with bulk-phase protons, must be omitted from the present analysis, but it is worth pointing out that the model derived in a somewhat different context by Schwarz [108–109] may well form a suitable starting point.

NOTE ADDED IN PROOF

Law and John [110] have recently studied the effects of lactoperoxidase-thiocyanate-peroxide (LPS) system on the electrochemical proton gradient in *Escherichia coli* cells, and found that although $\Delta\psi$ was inhibited by the LPS system ΔpH

was unaffected in intact cells. Similarly, the LPS system had no effect upon the H^+ permeability of these cells and did not inhibit the H^+-ATPase of this organism. These and other rapid effects of the LPS system on *E. coli* reviewed by Reiter [111], including a rapid loss of intracellular K^+ and pleiotropic inhibition of protonmotivated active transport systems, bear a striking similarity to the effects of membrane-active colicins on sensitive strains of *E. coli* reviewed above, and suggest that one of the primary target proteins of the LPS system in intact *E. coli* cells is the same as that of the membrane-active colicins. P. John (personal communication) has also drawn our attention to the effects of *Helminthosporium maydis* toxin on sensitive corn mitochondria, in which complete uncoupling of oxidative phosphorylation occurs at a toxin concentration of 10 pmol/mg protein [112]. It is not known ehether this toxin is protonophorous, but the existence of resistant mitochondria (from N cytoplasm corn) would seem to exclude this possibility. It seems feasible, therefore, that the molecular mechanism of uncoupling in this system is analogous to that exerted by the membrane-active colicins described above.

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