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MINI-REVIEW

Electron and Proton Transport Across the Plasma Membrane

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Abstract

Transplasma membrane electron transport in both plant and animal cells activates proton release. The nature and components of the electron transport system and the mechanism by which proton release is activated remains to be discovered. Reduced pyridine nucleotides are substrates for the plasma membrane dehydrogenases. Both plant and animal membranes have unusual cyanide-insensitive oxidases so oxygen can be the natural electron acceptor. Natural ferric chelates or ferric transferrin can also act as electron acceptors. Artificial, impermeable oxidants such as ferricyanide are used to probe the activity. Since plasma membranes contain *b* cytochromes, flavin, iron, and quinones, components for electron transport are present but their participation, except for quinone, has not been demonstrated. Stimulation of electron transport with impermeable oxidants and hormones activates proton release from cells. In plants the electron transport and proton release is stimulated by red or blue light. Inhibitors of electron transport, such as certain antitumor drugs, inhibit proton release. With animal cells the high ratio of protons released to electrons transferred, stimulation of proton release by sodium ions, and inhibition by amilorides indicates that electron transport activates the Na^+/H^+ antiport. In plants part of the proton release can be achieved by activation of the H^+ ATPase. A contribution to proton transfer by protonated electron carriers in the membrane has not been eliminated. In some cells transmembrane electron transport has been shown to cause cytoplasmic pH changes or to stimulate protein kinases which may be the basis for activation of proton channels in the membrane. The redox-induced proton release causes internal and external pH changes which can be related to stimulation of animal and plant cell growth by external, impermeable oxidants or by oxygen.

Key Words: Plasma membrane; NADH oxidase; transplasma membrane electron transport; proton release; Na^+/H^+ antiport; H^+ ATPase; transferrin; transferrin-stimulated oxidase; adriamycin; cis-platin, iron transport; blue light effects; auxin; coenzyme Q; turbo reductase; vitamin K; protein kinase; cell growth control.

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Introduction

There is a precedent for electron transport systems in eukaryotic plasma membranes. Transmembrane electron transport is clearly recognized and defined as to components and function in bacteria. Other prokaryotes (e.g., blue green algae) have plasma membrane electron transport, although not as well defined. For bacteria the importance is clear, since the plasma membrane has all of the energy coupling machinery for oxidative ATP synthesis. The situation is not as clear in prokaryotic algae, since they have thylakoid membranes, which have ATP-synthesizing machinery (Pescheck *et al.*, 1988). In most eukayotic cells the mitochondria handle the major high-efficiency ATP synthesis, so there is no need for an ATP-synthesizing system associated with plasma-membrane electron transport. If ATP can supply energy for all plasma membrane transport functions, and if transport is the only energy-requiring function of plasma membranes, then energy-coupled redox systems would be redundant in the outer membrane. There have been proposals for direct coupling of electron transport to ion or nutrient transport activity, but these have not developed clearly (Robertson, 1991). The question is: do eukaryotic plasma membranes have electron transport systems coupled to proton transport or ion movement and, if not, is the electron transport related to an energy-coupling process or does it serve another function? Clearly, the presence of masses of thiol groups on receptors requires electron transfer across the membrane to maintain the thiol state (Williams, 1989).

Other types of redox function are found in plasma membranes for special roles. The peroxide-generating NADPH oxidase in neutrophils used for killing bacteria (Mitchell, 1983), the xanthine oxidase which may have similar functions in addition to purine rescue (Tritsch and Niswander, 1983), and the cytochrome b_5-dependent fatty acyl CoA desaturase (Chmelar and Giacobino, 1976) are examples. Proton transfer across the plasma membrane by activation of a channel has been associated with the neutrophil transmembrane NADPH oxidase (Henderson *et al.*, 1988). This enzyme may serve as a model for other eukaryotic plasma membrane redox systems associated with proton release. The NADH-cytochrome b_5 reductase, fatty acid desaturase, or methemoglobin reductase are exclusively on the cytoplasmic side of the plasma membrane, so these enzymes have not been associated with any proton transport (Kant and Steck, 1972).

The Evidence for Transplasma Membrane Electron Transport

Evidence for transplasma membrane electron transport is found in all animal cells examined, ranging from protozoa to man (Ramirez, 1987;

Crane *et al.*, 1988a, 1990a; Dahse *et al.*, 1989). It is detected by reduction of impermeable dyes or complex ions by intact cells and by histochemistry (Morré *et al.*, 1978, 1987a). Electron transport reactions based on oxidation of NAD(P)H by oxygen or the same impermeable compounds can be detected in isolated plasma membranes in nonvesicular form (Sun *et al.*, 1987c). If the isolated membranes form sealed vesicles, then either the impermeable reductant or impermeable oxidant will not be available at its reaction site and the oxidoreductions observed will represent internal or external dehydrogenases, which may or may not be connected to the transmembrane enzyme. Insertion of substrate into vesicles followed by resealing has only been successful with electroporation of NADH into right side-out plant plasma membrane vesicles (Böttger, 1989). The NADH in these vesicles can then be oxidized by external ferricyanide. Ascorbate has been inserted successfully by other methods (Askerlund, 1990; Hassidim *et al.*, 1987).

Oxygen is permeable to membranes, so a transmembrane NADH oxidase can be measured if the plasma membrane vesicles are inside-out with the NADH dehydrogenase on the exposed face. On the other hand, impermeable hormones or other ligands for external surface sites will not be able to influence the oxidase reaction in the inside-out sealed vesicles. Fortunately, isolated liver plasma membranes do not vesiculate easily because of desmosomes (Morré *et al.*, 1987b). If liver plasma membranes are not homogenized too vigorously, they show NADH dehydrogenase activity, which is consistent with the transmembrane electron transport (Sun *et al.*, 1987c). Erythrocyte membranes must be prepared in the open ghost form to show transmembrane electron transport (Grebing *et al.*, 1984).

Plasma membranes also have sites for NADH oxidation on their external surface (Morré *et al.*, 1988a). NADH oxidase or ferricyanide reductase observed with right side-out vesicles will measure this outer surface activity, so it cannot represent the transmembrane activity seen with whole cells. It could, however, represent an external feed to a part of the transmembrane electron transport chain analogous to the external NADH site on mitochondrial cristae in plants (Møller and Lin, 1986).

Since substrate-loaded vesicles of proper orientation have not been obtained with animal plasma membranes, studies on proton transport accompanying the transmembrane electron transport have been restricted to intact cells.

With intact cells the shift in the redox state of $NADH/NAD^+$ when an external oxidant is reduced is consistent with the idea of a transmembrane electron transport (Navas *et al.*, 1988). It does not necessarily mean that NADH is the primary substrate since the NADH may reduce the primary substrate. For example, NADH may reduce semidhydroascorbate to ascorbate,

and ascorbate may be the primary electron donor to the transmembrane electron transport (Goldenberg *et al.*, 1983).

The Nature of Animal Cell Transmembrane Electron Transport

With intact cells or perfused tissue, the rate of transplasma membrane electron transport measured by ferricyanide reduction can be quite rapid (Löw *et al.*, 1990; Crane *et al.*, 1985a). For example, rat liver cells have ferricyanide reduction rates as high as 260 nmol per minute per g fresh weight of cells. If 4% of the liver cell protein is in the plasma membrane, then the rate of electron transport through the membrane would approach 100 nmol per minute per mg membrane protein. Rates of NADH-ferricyanide reductase up to 1000 nmol per minute per mg protein have been reported for erythrocyte membranes, and 780 nmol per min per mg for rat liver plasma membranes, but part of this activity will come from internal or external enzymes.

Of course, ferricyanide is not the natural electron acceptor, so these rates only give maximum electron transport capacities of the transplasma membrane enzyme.

Isolated rat liver plasma membranes have a unique NADH oxidase which is not sensitive to cyanide and is stimulated by azide, transferrin, and hormones (Gayda *et al.*, 1977; Morré and Crane, 1990; Crane *et al.*, 1985b; Thorstensen and Aisen, 1990). In isolated membranes the activity is up to 20 nmol per min per mg protein, unless it is stimulated by diferric transferrin or hormones. The measurement of the plasma membrane oxidase in cells is difficult because of the multifarious nature of cellular oxygen uptake. Since it is a cyanide-insensitive enzyme which may transfer electrons to impermeable external electron acceptors, transmembrane oxidase can be identified by measuring the effect of ferricyanide on cyanide-resistant oxygen uptake. The inhibition is postulated on the basis of ferricyanide-accepting electrons before the site where oxygen accepts electrons. In an experiment with well-oxygenated liver cells, it was found that 20% of the respiration was insensitive to 1 mM KCN and one-half of that oxygen uptake was inhibited by 0.2 mM ferricyanide. In the absence of cyanide, 0.1 mM ferricyanide inhibits oxygen uptake by rat liver cells 10%. The overall indication is that with well-aerated liver cells the transplasma membrane oxidase activity can be 10% of the total respiration (F. L. Crane, unpublished). Studies on the effect of diferric transferrin and growth factors on cyanide-resistant, ferricyanide-inhibited respiration of cells have not been done. There are many reports in the literature of complete inhibition of oxygen uptake by cyanide which actually should be unexpected, since internal endosomal cyanide-resistant oxidase (P450) are known in addition to the plasma membrane enzyme. If the plasma

membrane enzyme is involved in specialized functions or growth control, then it may be undetectable, unless diferric transferrin and growth factors are present. Transmembrane NADH-ferricyanide reductase activity is not necessarily connected to the oxidase activity, since open erythrocyte ghosts have no NADH oxidase activity, despite the high level of NADH-ferricyanide reductase (Morré and Crane, 1990; Crane *et al.*, 1985b). Mammalian erythrocytes also have no transferrin receptors and diferric transferrin does not activate NADH oxidase in these membranes (Sun *et al.*, 1987). However, if transferrin receptors are inserted into the erythrocyte membranes by *Falciparum* infection, then the cells show a transmembrane diferric transferrin reductase activity (Fry, 1989). Whether this is coordinated to induction of transmembrane oxidase and proton release remains to be seen.

The stimulation of the transmembrane oxidase by diferric transferrin brings up the question of how the transferrin acts. Does it act as a terminal oxidase by catalyzing the reoxidation of ferrous iron with oxygen as soon as the iron is reduced by the transmembrane electron transport (Bates *et al.*, 1973), or does binding of the diferric transferrin to the transferrin receptor activate the oxidase in the membrane by a conformational change in the redox system? In support of the terminal oxidase hypothesis, we have demonstrated that the transmembrane electron transport system can act as a ferric transferrin reductase (Löw *et al.*, 1986, 1987). Reduction of iron in diferric transferrin by cells can be demonstrated by direct spectrophotometric measurement of decrease in the absorbance of diferric transferrin at 465 nm under anaerobic conditions (unpublished), or by formation of ferrous bathophenanthroline disulfonate (BPS) in the media, when cells are incubated with diferric transferrin. BPS is an impermeable ferrous chelator. Reduction of the transferrin iron at the membrane can also be measured with formation of ferrous dipyridyl trapped in the membrane (Goldenberg *et al.*, 1988). The requirement for the transferrin receptor in these reactions with HeLa cells is indicated by inhibition with B3/25 and GB16 monoclonal antibodies to the transferrin receptor (Löw *et al.*, 1987).

NADH diferric transferrin reductase activity can also be demonstrated using isolated liver membranes (Sun *et al.*, 1987c). Three types of assay can be used to measure the activity. (1) Direct measurement of a decrease of the diferric transferrin at 465 nm absorbance in the presence of NADH and membranes under anaerobic conditions. The absence of oxygen is essential in this assay because the ferrous iron formed is immediately reoxidized by oxygen to re-form diferric transferrin. (2) Oxidation of NADH by membranes is greatly increased with diferric transferrin is added (Sun *et al.*, 1987c; Thorstensen and Aisen, 1990). This reaction can also be interpreted as a stimulation of an NADH-oxygen : oxidoreductase by diferric transferrin binding to the membrane. If the assay is for an NADH transferrin reductase,

then it should work under anaerobic conditions, which has not been tested. The requirement for the transferrin receptor in this reaction is likely, since the reaction does not occur in erythrocyte membrane (Fry, 1989). (3) Ferrous BPS is formed when diferric transferrin is added to liver plasma membranes with NADH. This type of assay has been criticized by Thorstensen and Aisen (1990) on the basis that the BPS effectivity raises the redox potential of the ferric transferrin to the point that it can be reduced by the transplasma membrane electron transport. Since diferric transferrin in simple solution at pH 7.0 has a redox potential at -500 mV and NADH has a potential at -320 mV, it is quite clear that they are correct that NADH cannot reduce diferric transferrin in simple solution. However, the presence of plasma membrane, transferrin receptor, and a complex transmembrane electron transport system introduces factors which do not allow a simple theoretical analysis of the possibility for reduction of external diferric transferrin by cytosolic NADH. Both the surface of cells and isolated membranes have a negative ζ potential, which can modify surface pH. Transferrin iron is released at pH below 7.0 and reduced by ascorbate ($+56$ mV). The redox potential of diferric transferrin bound to the transferrin receptor is unknown, and it may be much higher than free transferrin if the conformation of the transferrin is changed by binding. If a transferrin receptor site is not important, then reduction of ferric desferrioximine would also be expected (-430 mV) in the presence of BPS. This reduction is not seen with Hela cells (Lindgren, Crane, and Löw, unpublished). Finally, the reduction of NAD^+ by succinate in mitochondria would be impossible, except for the fact that the cristae membrane can carry out reversed electron transport energized by the proton gradient created by the electron-transport system. The plasma membrane may have an energy-linked reverse electron transport.

Actually, on thermodynamic grounds diferric transferrin reduction at the plasma membrane is even less likely than Thorstensen and Aisen (1990) propose because the redox potential of the electron carrier on the outer surface of the plasma membrane has been titrated at -160 mV (Sun *et al.*, 1984), which means that reduction of diferric transferrin at that site is less energetically favored than with NADH directly. The study of reduction of diferric transferrin by cells or membranes in the presence of BPS obviously will not answer the question whether diferric transferrin can be a natural acceptor for the plasma membrane electron transport. The fact that ferrous BPS formation occurs even in the Thorstensen and Aisen experiments is evidence for a transmembrane electron transport system, at least to high redox potential acceptors. Diferric transferrin in the presence of BPS can act as a high redox potential acceptor for the transmembrane electron transport. It should be noted that Thorstensen and Aisen (1990) do confirm diferric transferrin stimulation of the plasma membrane NADH oxidase. In their

studies they do not consider the direct measurement of diferric transferrin reduction by decline in absorbance at 465 nm under anaerobic conditions (Sun *et al.*, 1987c). The ESR evidence which they present as direct assay for reduction is by no means conclusive, since it is done in the presence of 1 mM BPS, which at this concentration acts as an inhibitor of the transmembrane (Crane *et al.*, 1985b). A more decisive answer could have been obtained if the experiment had been done under anaerobic conditions in the absence of BPS so that the loss of the transferrin iron signal could have been observed directly.

If not a redox carrier, then the diferric transferrin can act by binding to the transferrin receptor to activate the oxidase. The binding site at which diferric transferrin stimulates the NADH oxidase in the isolated plasma membrane appears to have much lower affinity for diferric transferrin than does the high-affinity binding site involved in iron uptake by endocytosis. Iron uptake is saturated at 1 μM diferric transferrin, whereas the stimulation of NADH oxidase by diferric transferrin is saturated at 40 μM (Crane *et al.*, 1990b). This low-affinity site is also involved in diferric transferrin reduction by cells and is probably the site involved in the "nonsaturable" iron uptake by liver (Trinder *et al.*, 1988). In other words, NADH oxidase stimulation and diferric transferrin reduction require 40 μM diferric transferrin to each saturation, which suggests that each of these activities occurs at the same site on the membrane. The inhibition of diferric transferrin reduction by intact HeLa cells with B3/25 and GB16 monoclonal antibodies but not by GB18 or 42/6 further indicates binding and reduction at a site different from the high-affinity binding site (Toole-Simms, 1988). GB18 and 42/6 bind an epitope at the high-affinity site on the transferrin receptor but B3/25 and GB16 bind elsewhere on the receptor. Cooperative effects of B3/25 and 42/6 on cell proliferation have been described (White *et al.*, 1990).

In conclusion, the relationship between the plasma membrane NADH oxidase and diferric transferrin appears to involve a direct stimulation of the NADH oxidase when transferrin binds to a low-affinity site on the transferrin receptor, as well as slow reduction of iron in the diferric transferrin. The slow reduction at the low-affinity site may add to the total oxidase activity by recycling the ferric-ferrous iron by oxidation on the transferrin after the ferric iron is reduced by transmembrane electron transport.

Components of the Transplasma Membrane Electron Transport System

Plasma membranes have been reported to contain flavin, cytochromes of the *b* type, nonheme iron, coenzyme Q, α-tocopherol, thiol groups, and possibly copper (Møller and Crane, 1990; Crane *et al.*, 1985b; Yamamoto

et al., 1985; Askerlund, 1990; Kalin *et al.*, 1987). Coenzyme Q is the only component for which there is good evidence for participation in transmembrane electron transport.

Evidence or Coenzyme Q Function

Reduction of ferricyanide and diferric transferrin are inhibited by analogs of coenzyme Q, and the inhibition is reversed by addition of coenzyme Q (Crane *et al.*, 1991a; I. L. Sun *et al.*, 1990). Piericidin A is the most effective inhibitor among the coenzyme Q analogs. 2,3-Dimethoxy-5-chloro-6-naphthyl-mercaptobenzoquinone and 2-methoxy-3-ethoxy-5-methyl-6-hexadecylmercaptobenzoquinone are also good inhibitors of diferric transferrin reduction by cells (Crane *et al.*, 1991a). The NADH-ferricyanide reductase and NADH oxidase activity of rat liver plasma membranes are inhibited by the same concentrations of the above inhibitors and addition of coenzyme Q_{10} partially restores the activity.

Extraction of coenzyme Q from lyophilized plasma membranes with heptane partially inhibits NADH-ferricyanide reductase activity. Activity is restored by addition of coenzyme Q in heptane membranes, followed by evaporation of the heptane by the Norling *et al.* (1974) procedure. Loss of activity is proportional to the amount of coenzyme Q removed (Crane *et al.*, 1990c, d; I. L. Sun *et al.*, 1990).

A precedent for coenzyme Q function is transmembrane electron transport is seen in mitochondria (Lenaz, 1985; Trumpower, 1982). A similar function as electron and proton carrier in the lipid phase may be possible in plasma membranes. It should be emphasized that the coenzyme Q appears to function before the site of external ferricyanide reduction by plasma membrane, whereas in mitochondria it functions after the site of ferricyanide reduction by NADH dehydrogenase. For example, piericidin A inhibits ferricyanide reduction in the plasma membrane, whereas it does not inhibit ferricyanide reduction by mitochondrial cristae (Hall *et al.*, 1966). Antimycin A and rotenone do not inhibit electron transport in plasma membranes (Clarke *et al.*, 1981; Barr *et al.*, 1985). Since they act at coenzyme Q binding sites in mitochondria, the coenzyme Q binding sites in the plasma membrane must differ from those in mitochondria (Crane, 1990).

Inhibitors of Transplasma Membrane Electron Transport

Inhibitors at specific sites in electron transport systems are useful in defining the sequence of the system or to see if the system contributes to a

celluar function. The transmembrane electron transport from cells or the NADH dehydrogenase activity of plasma membranes has been found to respond to some unique inhibitors.

For ferricyanide or diferric transferrin reduction by cells atebrin and chloroquine are effective at high concentrations (Sun *et al.*, 1987c; Löw and Crane, 1978; Toole-Simms *et al.*, 1990; Gayda *et al.*, 1977), whereas adriamycin, *cis*-dichlorodiamine platinum II, actinomycin D, and bleomycin inhibit at low concentrations (Sun and Crane, 1988). These same compounds are good inhibitors of NADH-ferricyanide reductase or NADH diferric transferrin with isolated plasma membranes. Atebrin and chloroquine are effective against malaria and the other compounds are used as antitumor agents, so the inhibitions may point to a vital role of the redox sysem in cancer and infections by protozoa.

The important antitumor drugs which are strong inhibitors of trans-plasmalemma electron transport include adriamycin and related anthracyclines —bleomycin, *cis*-diaminodichloroplatinum II (cis-platin), actinomycin D, anthramycin, and retinoic acid (Sun and Crane, 1988, 1990). Electron transport by transformed cells or tumor cells is more sensitive to these compounds than with normal cells and inhibition occurs at concentrations which inhibit cell growth (Sun *et al.*, 1986b). Except for retinoic acid (Sun *et al.*, 1988b), proton release associated with the redox activity is also inhibited at the same concentrations starting at 10^{-7} M (Sun and Crane, 1988). Adriamycin coupled to transferrin with glutaraldehyde is more effective than adriamycin alone in inhibition of transmembrane electron transport and redox-induced proton release. Good inhibition is seen with HeLa cells at 10^{-8} M adriamycin equivalent. Since the effect is seen in 3 minutes, the effectiveness of the conjugate suggests that the adriamycin acts at the plasma membrane and the redox enzyme is close to the transferrin receptor (Faulk *et al.*, 1988, 1989, 1990). The time is too short for the conjugate to release adriamycin to the nucleus.

Proton Release Associated with Transplasma Membrane Electron Transport: Animal Cells

Transplasma membrane electron transport is associated with proton release from cells, as measured by a change in the external pH (Dormandy and Zarday, 1965; Sun *et al.*, 1984; Sun and Crane, 1984). Reduction of both ferricyanide and diferric transferrin is accompanied by proton release. The ratio of proton release to electron transfer is much lower for ferricyanide than for diferric transferrin (Table I). Since ferrocyanide and apotransferrin do not stimulate proton release, an electron acceptor is necessary. Inhibitors of

Table I. Ratio of Proton Release to Ferricyanide and Diferric Transferrin Reduced by Animal Cell Lines

Media	Cell	Ferricyanide reduction (neq min^{-1})(gww^{-1})	H^+ release	Ratio H^+/e^-	References
10 mM NaCl	Ehrlich ascites	15.6	52	3.3	Waranimman *et al.*, 1986
250 mM sucrose	$SV40_{ts}$ pineal 33°	65	208	3.2	Sun *et al.*, 1986b
250 mM sucrose	$SV40_{ts}$ pineal 40°	192	308	1.6	Sun *et al.*, 1986b
150 mM NaCl	$SV40_{ts}$ pineal 33°	101	540	5.3	Sun *et al.*, 1988a
250 mM sucrose	$SV40_{ts}$ pineal 33°	—	81	—	
150 mM choline Cl	$SV40_{ts}$ pineal 33°	34	0	0	Sun *et al.*, 1986a
10 mM NaCl	$SV40_{ts}$ rat liver 33°	552	36	15.3	Sun *et al.*, 1986a
10 mM NaCl	$SV40_{ts}$ rat liver 40°	558	88	6.7	Sun *et al.*, 1986a
		Diferric transferrin reduction			
150 mM NaCl	$SV40_{ts}$ pineal 33°	16.5	1530	93	Sun *et al.*, 1988a
150 mM choline	$SV40_{ts}$ pineal 33°	5.6	0	0	Sun *et al.*, 1988a
140 mM NaCl	Hela	31[a]	1107	36	Toole-Sims, 1988

[a] Reduction rate may include loosely bound iron measured by the ferrous bathophenanthroline formation.

transplasma membrane electron transport, such as adriamycin (Sun and Navas, 1987; Sun and Crane, 1984), bleomycin (Sun and Crane, 1985), cis-platin, and piericidin A (Crane *et al.*, 1990c), as well as monoclonal antibodies to the transferrin receptor (Toole-Simms, 1988), inhibit the proton release at the same concentrations which inhibit electron transport; the redox system appears responsible for activation or driving the proton movement.

There are several known mechanisms by which proton transfer across the membrane could be coupled to the transplasma membrane electron transport. It could be based on (a) anisotropic arrangement of protonated and nonprotonated electron carriers as proposed by Mitchell (1983) for mitochondria, or (b) the electron transport protein could act as a redox-controlled proton channel as proposed by Wikström (1981), Wikström and Krab (1979), and Wikström *et al.* (1981), for cytochrome oxidase, or (c) the Q cycle with oxidation and reduction of coenzyme Q on opposite sides of the membrane might apply to plasma membrane, since coenzyme Q is present in the membrane (Kalin *et al.*, 1987).

As an alternative, the redox-generated proton release could be based on activation of a channel or pump, such as the Na^+/H^+ antiport or a proton-pumping ATPase.

The analysis of how redox-induced proton release occurs in the plasma membrane is far from complete. In early studies with ferricyanide as an electron acceptor, the stoichiometry of protons released to ferricyanide reduced was around 2 or 3, which would be consistent with proton transfer through redox carriers during their oxidation–reduction cycle (Sun *et al.*, 1984). Later studies find 5 to 15 protons released per ferricyanide reduced, which would be more appropriate for activation of a channel (Table I). Evidence that the Na^+/H^+ antiport could be the channel activated by ferricyanide was developed by Garcia-Cañero *et al.* (1987), when they showed that ferricyanide reduction stimulated Na^+ uptake by liver cells. They also showed Na^+ dependence and amiloride inhibition of the ferricyanide reduction. With HeLa cells the ferricyanide-induced electron transport was inhibited by amiloride and increased in Na^+-containing media (Sun *et al.*, 1987b). Fuhrmann *et al.* (1989) have also reported Na^+ influx into erythrocytes in presence of 5 mM ferricyanide.

The lack of inhibition of proton release by 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) and 4-acetamido-4′isothiocyanatostilbene-2,2′-disulfonic acid (SITS) treatment of cells indicates that the HCO_3^-/Cl^- anion exchanger is not the basis for ferricyanide-induced proton transfer (Toole-Simms, 1988).

Diferric transferrin reduction is accompanied by a much greater proton release than with ferricyanide (Table I). These are transformed cells which would tend to have high levels of transferrin receptor, which may optimize

the transferrin-related redox function. The stoichiometry of proton release to ferrous BPS formation as a measure of diferric transferrin reduction is often over 100. An H^+/e^- ratio this large would be consistent with activation of a H^+ channel rather than a carrier-dependent H^+ transfer.

These observations are subject to two major caveats: (1) The transferrin-stimulated NADH oxidase has not been measured as part of the diferric transferrin-stimulated electron transport, so the ferrous BPS formation may represent only a part of the electron transfer which is inducing H^+ release. (2) Some preparations of diferric transferrin have adventitious loosely bound iron, which greatly stimulates the rate of ferrous BPS formation by cells. An indication of the effect of extra iron is seen where ferrous BPS formation is 140 nmol min^{-1} gww^{-1} for HeLa cells with 10 μM diferric transferrin. The addition of 10 μM apotransferrin to convert all iron to the tightly bound form decreases the reduction rate to 80 nmol min^{-1} gww^{-1}. Further decrease may be achieved by incubating the ferric transferrin with apotransferrin before starting the reaction (Löw *et al.*, 1988; Crane *et al.*, 1990b).

An extensive series of studies on redox-induced proton release by rat pineal cells in the transformed and untransformed phenotype based on temperature-sensitive SV40 (Sun *et al.*, 1988a) is consistent with dependence of a major part of the proton release on the Na^+/H^+ antiport activation with a small part possibly dependent on some other pathway (Toole-Simms, 1988).

Inhibition of Proton Release

Good evidence for the requirement for electron transport to activate the antiport is seen in the specific inhibition of oxidant-induced proton release by agents which inhibit the transplasma membrane electron transport. These agents include adriamycin, cis-platin, bleomycin, and actinomycin D (Sun and Crane, 1988, 1990), as well as inhibitory coenzyme Q analogs, piericidin A, and 2-methoxy-3-ethoxy-5-methyl-6-hexadecylmercapto-1,4-benzoquinone. The effects of the coenzyme Q analogs are reversed by added coenzyme Q (Crane *et al.*, 1990b).

Retinoic acid is a special case. It inhibits transmembrane electron transport without inhibition of proton release (Golub *et al.*, 1988). Retinoic acid also stimulates proton release in the absence of oxidants or other agonists to activate the antiport, so the retinoic acid, which is permeant acid, may activate the antiport by direct acidification of the allosteric site (Ladoux *et al.*, 1987). The continued proton release with retinoic acid, even with inhibition of transmembrane electron transport, is in contrast to the inhibition of both functions by adriamycin and other antitumor drugs. This difference

may relate to the ability to retinoic acid to induce differentiation of transformed cells (E. Sun *et al.*, 1990).

The lack of retinoic acid inhibition of electron transport in SV40 transformed cells is further evidence that the portion of large T antigen inserted into the plasma membrane modified the electron-transport system (Sun *et al.*, 1988b).

How Does Electron Transport Activate the Antiport?

The mechanism for activation of the Na^+/H^+ antiport by the transmembrane electron transport is not known. There are logical consequences of electron transport or some experimental observations which suggest mechanisms for transfer of a stimulus from the redox system to the antiport based on the current ideas concerning the site of activation on the antiport itself. These mechanisms could be (1) activation of a protein kinase to phosphorylate the antiport, (2) localized proton increase as a result of oxidation of a protonated electron transport carrier (e.g., coenzyme Q) with subsequent direction of the proton to the allosteric activation site on the antiport through a closed channel, (3) changes in pH set point of the antiport by conformational change in a closely associated redox protein during oxidation–reduction (Grinstein *et al.*, 1989), or (4) reduction of disulfide bonds which control antiport activity (Fuhrmann *et al.*, 1989; Boniface and Reichert, 1990).

The evidence that the antiport is regulated by phosphorylation on a serine (Sardet *et al.*, 1990) opens up a new approach to control of the antiport through the plasma membrane redox system. Tyrosine kinase can activate serine/threonine kinase (PKC or MAP). Isolated tyrosine kinase is activated by low levels of H_2O_2 (Gopalakrishna and Anderson, 1989). Low *et al.* (1990) have shown that band 3 in erythrocytes (note that the erythrocyte antiport at 110 kDa should be included in the band 3 proteins) is phosphorylated when H_2O_2 is added to the cells and that external ferricyanide can also cause phosphorylation of band 3. H_2O_2 has long been known to increase phosphorylation of other membrane proteins, e.g., the insulin receptor to mimic the action of insulin (Koshio *et al.*, 1988). Quinones, such as coenzyme Q, can generate H_2O_2 in membranes by autooxidation of semiquinones formed during the electron transport (Beyer *et al.*, 1987; Crane, 1990; Lenaz, 1985). Since there is now evidence that coenzyme Q functions in the plasma membrane electron transport (Crane *et al.*, 1991a, 1990d), and H_2O_2 generation occurs during NADH oxidation with isolated liver plasma membrane (Ramasarma *et al.*, 1981), one must consider if generation of a low level of H_2O_2 is the basis for antiport activation by the plasma membrane redox system (Low *et al.*, 1990).

The redox state of a quinone in a membrane has been shown to control protein kinase activity. The redox state of platoquinone in chloroplasts controls phosphorylation of the light-harvesting complex protein (Anderson and Andersson, 1988).

Addition to permeant acids to cells also activates the antiport (Grinstein *et al.*, 1989). The protons are considered to act at an allosteric activator site on the cytosolic domain of the antiport which may be associated with the phosphorylated site. Oxidation of NADH on the cytosolic side produces protons which would be close to the allosteric activator site. Oxidation of cellular NADH by external ferricyanide and diferric transferrin has been shown (Navas *et al.*, 1988). If the protons from the redox activity are released into a closed channel, which communicates with the activator site, then activation by redox action could occur without decreasing the bulk cytosolic pH. A channel of this type, controlled by calcium, has been described in chloroplasts (Dilley and Chiang, 1988).

The possible relation between the transmembrane electron transport system, the transferrin receptor, and the Na^+/H^+ antiport is diagrammed in Fig. 1. Redox activation of a proton channel has also been described during the respiratory burst of erythrocytes, where rapid H_2O_2 formation occurs (Henderson *et al.*, 1988). A role of protein kinase C in this process has also been indicated (Heyworth and Badwey, 1990).

Transplasma Membrane Electron Transport in Plants

There is extensive agreement that electrons are transferred across the plant plasma membrane to reduce oxidants of relatively high redox potential in the apoplast or outside the cell (Crane *et al.*, 1988, 1991; Lüthje and Böttger 1989; Møller and Crane, 1990). Part of this electron transfer may be mediated by export of reducing substances, such as caffeic acid of other phenols, through the plasma membrane. Generation of superoxide in the membrane which can reduce external oxidants has also been demonstrated (Cakmak *et al.*, 1987). The major part of the electron transport appears to be through a transmembrane oxidoreductase system which oxidizes cytosolic NADH or NADPH (Fig. 2) (Krüger and Böttger, 1988). The activity is most easily demonstrated with artificial impermeable probes, such as hexacyanoferrate III (ferricyanide), hexahalogen iridates IV, or hexaamine ruthenium (Lüthje and Böttger, 1989). The rate of reductant excretion is generally much less than the rate of electron transport to oxidants. Superoxide formation cannot explain all the activity, since only part to the reduction is inhibited by superoxide dismutase (Barr *et al.*, 1985) or removal of oxygen (Coleman and Chalmers, 1988). The natural oxidants for the

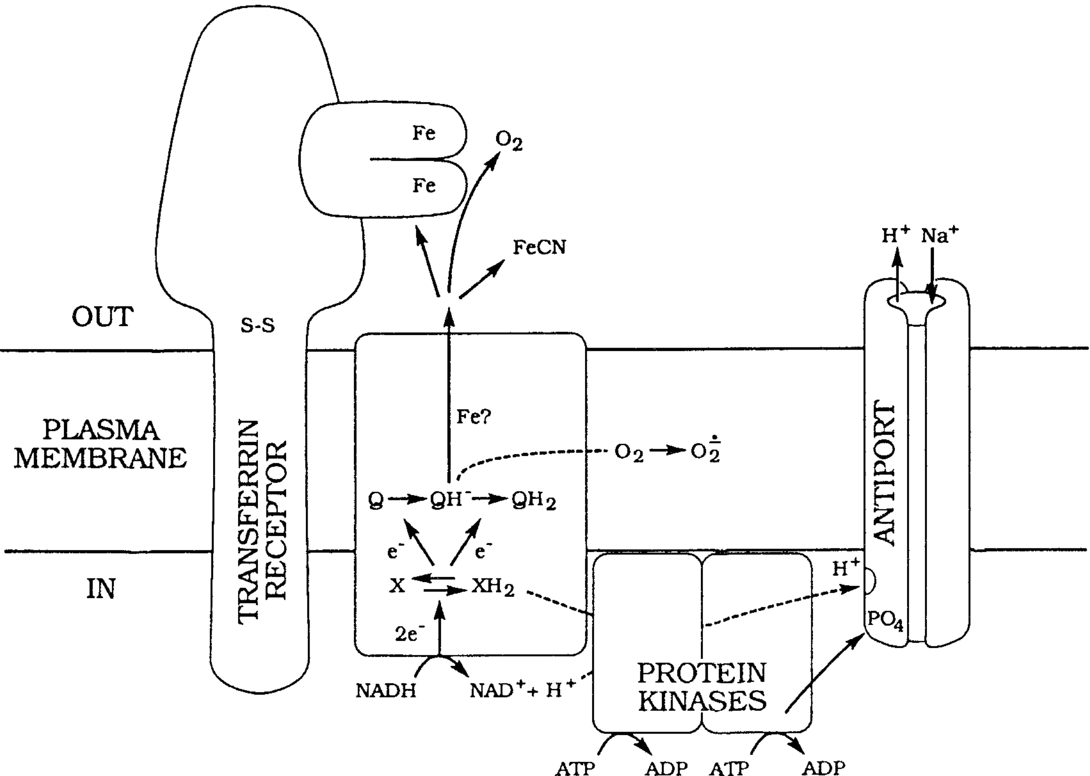

Fig. 1. Diagram showing the proposed relation between the transplasma membrane electron transport system, the transferrin receptor, and the Na^+/H^+ antiport for mammalian cells. Electron transport across the membrane is stimulated by ferric transferrin associated with the transferrin receptor. As a consequence of the electron transport activity, the antiport is activated either by proton release from protonated electron carriers or by generation of peroxide from superoxide to activate protein kinases to phosphorylate the antiport. Oxidation of a coenzyme Q semiquinone is the most likely source of superoxide. External NADH may also be oxidized by the redox enzyme (not shown). X may be a flavoprotein.

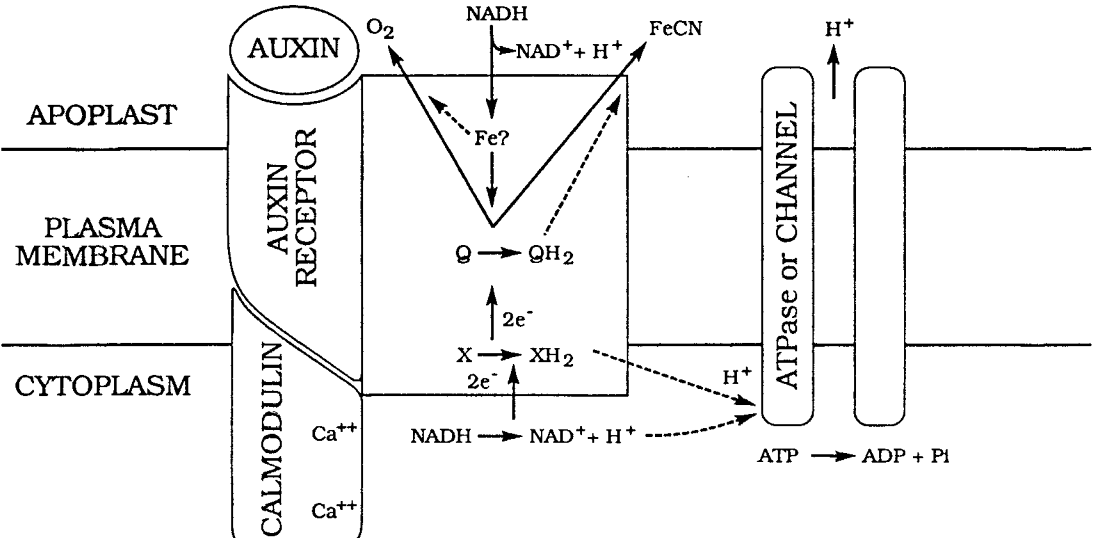

Fig. 2. Diagram of the proposed relation between the auxin-activated NADH oxidase in plant plasma membrane and the H^+ ATPase. Binding of auxin to the receptor activates the oxidase and the activation is controlled by a calmodulin. Oxidation of NADH activates the proton pump. The activation may occur by proton release, membrane potential change, or generation of superoxide at the quinone site to activate a kinase. X may be a flavoprotein and Q may be a vitamin K.

transmembrane enzyme can be oxygen, iron II complexes, or possibly other transition elements such as manganic ions (Jones, *et al.*, 1987).

Despite the high rates of transmembrane electron transport observed with ferricyanide or the hexahalogeniridates with intact cells, it has been difficult to demonstrate transmembrane electron transport using isolated plasma membranes. Askerlund *et al.* (1988) compared the NADH-ferricyanide reductase activity of open and closed membrane vesicles and concluded that transmembrane activity which would be revealed only with open membranes would constitute only 10% of the total NADH dehydrogenase activity in the membrane. Experiments with sugarbeet leaf inside-out sealed vesicles, with NAD^+ and alcohol dehydrogenase incorporated inside to generate NADH when ethanol was added, showed no reduction of external ferricyanide, cytochrome *c*, of ferric citrate but did reduce dichloroindophenol sulfonate (DCIPS) (Askerlund, 1990). Giannini and Briskin (1988) used freeze–thaw treatment to incorporate NADH into right side-out beetroot vesicles. They found no ferricyanide reduction, unless detergent was added to allow the ferricyanide to react with internal NADH hydrogenase. Böttger (1989) incorporated NADH into right side-out soybean hypocotyl plasma membrane vesicles by electroporation. Very slow rates of transmembrane electron transport to ferricyanide were found, unless protonophores were added. Electron transport was favored when the internal pH of the vesicles was 5 and inhibited at higher pH. The interpretation was that oxidation of internal NADH through a series of carriers would remove protons from the vesicle and that protonophores, such as FCCP, would allow proton supply from the outside to keep the reaction going (Böttger and Crane, unpublished; Barr *et al.*, 1990a) (Table II).

Oxidation of the internal NADH by oxygen was also demonstrated with these electroporated vesicles, and the reaction showed a pH dependence similar to ferricyanide reduction, so NADH oxidase action may also deplete protons from the interior of the vesicle (Böttger and Crane, unpublished).

Cis Electron Transport on Plasma Membranes

In addition to transplasma membrane electron transport, the plant plasma membranes also have NADH dehydrogenase enzymes which transfer electrons to acceptors on the same side of the membranes, referred to as cis electron transport. The enzyme on the inside is the NADH-cytochrome b_5 reductase (cytochrome b_5), which is common on endomembranes. The external enzyme can reduce ferricyanide and oxygen, and it may have an interconnection to the transmembrane enzyme analogous to the inner and outer dehydrogenases in plant mitochondria. The external NADH oxidase

Table II. Transplasmalemma Electron Transport from Plant Cells[b] and Sealed Right Side-Out Plasma Membrane Vesicles

Cell type	Electron acceptor	Reduction rate (nmol min^{-1} gww^{-1})	References
Corn roots	FeCN	50	Qui *et al.*, 1985
Corn roots	FeCN	20	Federico and Giartosio, 1983
Pea leaves	FeCN	19	Weber and Lüttge, 1988
Oat roots	FeCN	16	Rubinstein *et al.*, 1984
Carrot cells	FeCN	50	Barr, 1987
Oat leaves	FeCN: Light	60	Dharmawardhane *et al.*, 1987
	Dark	25	
Corn roots	HBI	18	Lüthje and Böttger, 1989
Sugar beet leaves	FeCN	47[a]	Askerlund, 1990
Plasma membrane vesicles	**Electron acceptor**	**Reduction rate (nmol min^{-1} mg $protein^{-1}$)**	**References**
Soybean hypocotyl NADH-loaded vesicles	FeCN	10	Böttger and Crane, unpublished
			Böttger, 1990
NADH + ADH,[c] beet leaves	FeCN	0	Askerlund, 1990
NADH + ADH, beet leaves	DCIPS	5.3	Askerlund, 1990
NADH, beet root	FeCN	0	Giannini and Briskin, 1988
Ascorbate, radish			Hassidim *et al.*, 1987
Ascorbate, beet leaves	Ferric citrate	2.9	Askerlund, 1990
Ascorbate, beet leaves	DCIPS	5.0	Askerlund, 1990

[a] Rate with sugar beet leaf segments was the same in light and dark.
[b] Further reference, Dahse *et al.*, (1989).
[c] ADH, alcohol dehydrogenase; DCIPS, dichloroindophenol sulfonate (an impermeable dye); FeCN, ferricyanide; HBI, hexabromoiridate IV.

has been shown to activate K^+ transport when NADH is added to the outside of plant cells or roots. The cytochrome b_5 reductase is not a transmembrane enzyme and is not known to affect ion movement (Askerlund *et al.*, 1988). Peroxidases in the apoplast can also oxidize added NAD(P)H, and their relation to NAD(P)H oxidase activity in the plasma membrane has been widely discussed and reviewed (Crane and Barr, 1989).

The Turbo Reductase

Iron deficiency induces very high transplasma membrane electron transport activity in the roots of many dicots and some monocots. This induced activity has been called the turbo reductase (Bienfait, 1985) because of its high activity. It appears that the turbo enzyme is different from the constitutive transmembrane enzyme in that it favors the reduction of FeIII chelates and may be able to reduce compounds at a lower redox potential than zero millivolts, which is the limit for the standard system (Bienfait, 1988a,b). It is not known if the turbo system arises by modification of the standard enzyme or by synthesis of a new enzyme system. Bruggeman *et al.* (1990) could not identify a new NADH-EDTA reductase in iron-deficient tomato roots, concluding that the standard enzyme may be increased. Since ferrous iron inhibits H^+ ATPase and proton release after turbo enzyme induction, ferrous ions may act as a messenger to suppress the activity (Zocchi and Coscucci, 1990).

Possible Electron Carriers in the Plasma Membrane

Isolated plant plasma membranes have been reported to contain flavin, iron, manganese, copper, and cytochromes of the *b* type (Møller and Crane, 1990). In contrast to animal membranes, they do not contain significant amounts of coenzyme Q, but material with a spectrum like vitamin K has been detected. Vitamin K_1 and menadione stimulate transmembrane electron transport, and dicoumarol inhibits, which is consistent with vitamin K function (Barr *et al.*, 1990c). Inhibition with menadione has also been reported (Bernstein *et al.*, 1989). A major and minor cytochrome in plasma membranes have a maximum at 20°C at 560 nm and a single peak at 557 nm at 77 K. The major (77%) component is reduced by ascorbate and has a redox potential around 150 mV (Caubergs *et al.*, 1988). This cytochrome also participates in the light-induced absorbance change system of flavin–cytochrome *b* complex that is reduced by blue light. A small amount of cytochrome b_5 with a split absorption peak at 552 and 558 nm at 77 K and a redox potential around

− 50 mV is also present (Askerlund, 1990). The presence of nonheme-bound iron has also been detected by ESR (Askerlund, 1990). Boron is required for ferricyanide-induced proton release from carrot and tomato cells (Goldbach *et al.*, 1990).

Plant plasma membranes thus contain more than enough prosthetic groups to account for a transplasma membrane electron transport system. The sequence of function remains to be determined.

Proton Movement Associated with Plant Plasma Membrane Redox Activity

Many plant cells release protons to produce an external, apoplast pH which is 1–2 pH units lower than that of the protoplast. The maintanence of this proton gradient requires energy transduction. The major part of the proton excretion can be attributed to an outwardly directed ATPase which has been well characterized (Serrano, 1985; Sze, 1985). There is evidence, however, that under some conditions the transplasma membrane electron transport system can stimulate or support proton release (Rubinstein and Stern, 1986; M. T. Marré *et al.*, 1988).

The most direct demonstration that oxidoreductase activity can control proton release is that ferricyanide increases proton release when it is reduced by plant cells. Since ferricyanide is not a physiological electron acceptor, the question arises if there arises if there is a natural acceptor for the electron flow which stimulates proton release. Oxygen, iron chelates, and nitrate have been suggested to be the natural oxidants (Morré *et al.*, 1988; Bienfait and Lüttge, 1988; Jones and Morel, 1988).

Proposed mechanisms for oxidant-induced proton release from plant cells fall into two major categories:

A. Activation of the proton-pumping plasma membrane ATPase (Rubinstein and Stern, 1986; E. Marré et al., 1988; Goldbach et al., 1990). It is abundantly clear that the major proton release by plant cells is carried out by the H^+-ATPase. The question is whether the plasma membrane electron transport can activate the ATPase to release more protons and, if so, how it initiates activation.

The evidence is that when ferricyanide or other impermeable oxidants are reduced by plant cells, the rate of acidification of the media increases. Oxidation of NAD(P)H in the cell may release protons to acidify the cytosol (E. Marré *et al.*, 1988). Acidification of the cytosol, e.g., by the addition of permeable acids, activates the H^+ ATPase. Therefore, if the transmembrane electron transport system only transfers electrons and leaves the protons inside, it could acidify the cytoplasm and activate the H^+ ATPase. Observations during ferricyanide reduction are consistent with this explanation.

^{31}P NMR shows cytosolic acidification. The interior of the cell becomes more positive to hypopolarize the membrane. Ferricyanide can also change the NADH/NAD ratio in the cytosol which can modify the H^+ ATPase activity (Elzenga *et al.*, 1989).

In many cells, inhibitors of the H^+ ATPase partly inhibit the ferricyanide-stimulated H^+ release (Rubinstein and Stern, 1986; Goldbach *et al.*, 1990). Activation of the H^+ ATPase may be caused by protonation of an allosteric site on the ATPase. As an alternative, there may be a configurational change in the redox system, or the electron transport may activate a protein kinase to phosphorylate the ATPase or an associated protein. Boron is also required for auxin stimulation of ferricyanide-induced proton release (Goldbach *et al.*, 1990). There is precedent for transmembrane electron transport or for oxidation–reduction of quinone in a membrane inducing activation of a protein kinase (Caughlin and Hind, 1987).

B. Anisotropic electron and proton transfer. If electron flow across the membrane involves protonated electron carriers or activates proton channels in protein involved in the electron transport, then the acidification of the external media could be based directly on the electron transport system. If so, the plasma membrane redox system would, in principle, be similar to the anisotropic mitochondrial electron transport system. Whether a complex system like that of mitochondria is feasible in the plasma membrane might be doubtful, based on the paucity of known electron carriers in the plasma membrane.

Proposals have been made to account for acidification of the media and depolarization of the membrane during ferricyanide reduction by direct action of an anisotropic electron transport. These proposals depend primarily on natural electron transport to oxygen, which is perturbed by competition with ferricyanide. The simplest proposal of this type (Novak and Ivankina, 1978; Böttger and Lüthen, 1986) would depend on transfer of electrons and protons from NADH to an external protonated carrier, e.g., a quinone, which would transfer electrons to an internal carrier to reduce oxygen and would release protons to the media. This would remove net positive charge to hyperpolarize the membrane. When ferricyanide is added, the electrons would be removed with the protons so the normal hyperpolarizing oxidase would be replaced by an electroneutral ferricyanide reduction and it would appear that the ferricyanide caused a depolarization of the membrane, because of loss of the unbalanced electron flow.

Observations have been made which support the presence of a hyperpolarizing oxidase activity in the membrane which ferricyanide can compete with (Böttger *et al.*, 1991; Ivankina and Novak, 1978). It should be emphasized that NADH oxidase activity in isolated plasma membrane can be rather slow unless it is stimulated by auxin (Morré *et al.*, 1986; Brightman *et al.*,

1988, so the influence of this oxidase on membrane potential and external acidification may amount to very little in resting plant cells. On the other hand, this type of oxidase could explain much of the proton release induced by auxin treatment.

1. Proton release by plant tissue is stopped at rather high oxygen tension compared to mitochondrial respiration and ATP synthesis. The plasma membrane oxidase requires 40 μM O_2 for saturation compared to 1–4 μM for mitochondria (Böttger *et al.*, 1985; Böttger and Lüthen, 1986).

2. 2-Deoxyglucose prevents ATP formation and decreases ATP to very low levels but maintains proton release at 50% of normal. If 2-deoxyglucose is metabolized by the hexose monophosphate shunt to produce NAD(P)H, the oxidation of NAD(P)H by the plasma membrane oxidase could explain the proton release observed (Xia and Saglio, 1990).

3. Inhibitors of mitochondria ATP production, e.g., cyanide, antimycin a, oligomycin, do not inhibit ferricyanide-activated H^+ release (Barr *et al.*, 1987; Barr, 1988).

4. In some cells inhibitors of H^+ ATPase activity only partly inhibit ferricyanide-induced proton release (Neufeld and Bown, 1987; Bown and Crawford, 1988).

5. Membrane potential is not directly proportional to cellular ATP levels, so an electrogenic redox pump may also be involved when artificial oxidants are not present (Löppert, 1983).

6. Artifical external oxidants can inhibit oxygen uptake. This is especially true when oxygen uptake is stimulated by ethanol (NADH) or auxin (Polevoy and Salamatova, 1977; Crane *et al.*, 1988b). In some tissue ferricyanide increases respiration, which would be expected if cytosolic NADH is oxidized to stimulate glycolysis. Only when the NADH pool is high would the ferricyanide inhibit respiration, based on the plasma membrane NADH oxidase. Residual respiration is inhibited by ferricyanide.

7. Artificial electron acceptors can restore proton release at low oxygen concentrations (Böttger *et al.*, 1985; Böttger and Lüthen, 1986).

8. In roots, auxin inhibits both proton secretion and ferricyanide reduction (Böttger and Hilgendorf, 1988).

The relation between the possible redox-driven proton transport and activation of the H^+ ATPase in causing observed changes in membrane potential is not clear, since each could contribute to the membrane potential. In addition, K^+ movement out of the cell through membrane channels along its concentration gradient can also contribute to membrane potential. The depolarizing effect of ferricyanide may be related to the effect of the redox system on any of these electrogenic functions: anisotropic electron tansport, H^+ pumping ATPase, or control of K^+ channels (Prins and Elzenga, 1991; Lucas and Kochian, 1988) (Fig. 3).

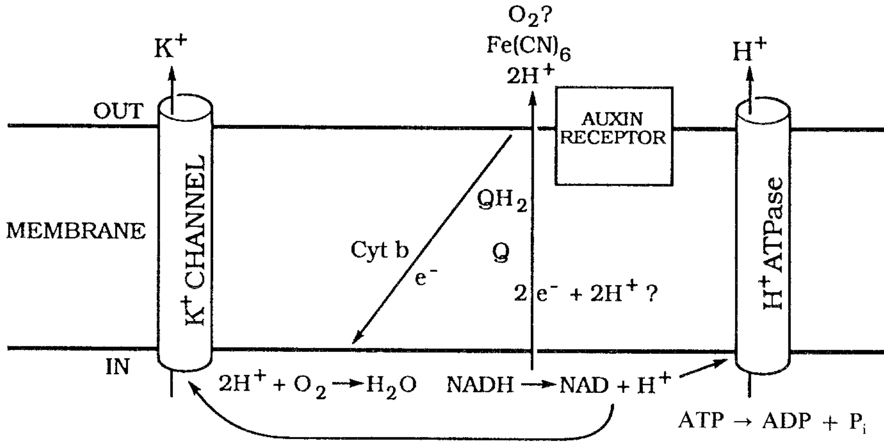

Fig. 3. A diagram to show possible interactions between the plasma membrane electron transport in plants in an anisotropic looped arrangement and the other main cation transporting systems, the H^+ ATPase and K^+ channels. Evidence for activation of either channel has been found when external ferricyanide is reduced. If there is a protonated redox carrier in the membrane, such as vitamin K, then oriented proton movement could accompany electron flow. Otherwise, electron transport across the membrane would acidify the cytoplasm to activate the H^+-ATPase. Ferricyanide reduction also can increase K^+ release from the cytoplasm through K^+ channels in the membrane (Lucas and Kochian, 1988).

Ferricyanide-induced K^+ efflux has been observed in several species, and the total H^+ plus K^+ release from cells is often equivalent to the ferricyanide reduced, so that the ratio $H^+ + K^+/e^-$ equals 1 (Fig. 3) (Ullrich and Guern, 1990).

Ullrich *et al.* (1990) find that neutral red (E_0 at pH 7 $= -320\,mV$) can act as an acceptor for transmembrane electron flow, which causes internal acidification and K^+ release which would indicate that the H^+-ATPase is not activated under these conditions.

Auxin Control of Plasmalemma Electron Transport and Proton Release

Auxins, or plant hormones which stimulate elongation growth of meristematic cells, and cell proliferation in some tissues, increase proton release from cells (Hager *et al.*, 1971; Cleland, 1976; Cleland and Rayle, 1978). Active compounds include indoleacetic acid, α-naphthalene acetic acid, and 2,4-dichlorophenoxyacetic acid. A proton-pumping ATPase in the plasma membrane is required for the auxin-induced proton release (Serrano, 1985; Sze, 1985). Auxin receptors have been identified on the plasma membrane (Löbler and Klämbt, 1985; Venis, 1985) as well as in internal

membranes, and direct action of auxin on DNA to promote messenger RNA synthesis has been proposed (Theologis, 1989). The mechanism of auxin is not clear. Considerable evidence indicates that auxin may act by stimulation of the plasma membrane NADH oxidase to activate proton release.

1. Auxins specifically stimulate plasma membrane NADH oxidase on intact cells, isolated membrane, or on a purified NADH oxidase from plasma membrane (Brightman *et al.*, 1988). Auxin also increases ferricyanide-stimulated proton release (Goldbach *et al.*, 1990).

2. The auxin effect on the oxidase occurs within 1 minute and is seen with isolated membranes, so it cannot come from activation of a nuclear site (Brightman *et al.*, 1988).

3. Antibodies to the auxin receptor isolated from plasma membrane inhibit the NADH oxidase (Morré *et al.*, 1989a).

4. Auxin-stimulated oxygen uptake by cells is inhibited by ferricyanide (Polevoy and Salamatova, 1977).

5. Auxin in high concentrations (10 μM) inhibits transmembrane ferricyanide reductase activity (Barr *et al.*, 1990b).

6. Auxin increases plasma membrane potential (Cleland *et al.*, 1977).

7. Ferricyanide can decrease plasma membrane potential (Elzenga and Prins, 1987).

8. Specific inhibitors of the plasma membrane NADH oxidase, e.g., actinomycin D, adriamycin, or cis-platin, also inhibit elongation growth of stems at the same concentrations that inhibit the oxidase (Morré *et al.*, 1988).

9. Auxin can cause acidification of the cytoplasm as does ferricyanide reduction (Brummer *et al.*, 1984; Rubinstein and Stern, 1986; Qiu *et al.*, 1985; Guern *et al.*, 1988).

10. Ferricyanide reduction by cells activates proton release (Craig and Crane, 1981; Federico and Giartosio, 1983). Low concentrations of ferricyanide stimulate elongation growth of roots (Lüthje and Böttger, 1989).

11. Anaerobic conditions inhibit H^+ release from carrot cells 92% in absence of ferricyanide but only 60% in presence of ferricyanide (Barr, 1987).

These effects are consistent with the presence of a branched transmembrane electron transport system in the membrane, where the electron flow to oxygen is increased by auxin, whereas electron flow to the ferricyanide reduction site is inhibited by auxin. Ferricyanide and oxygen would compete for electrons. Electron flow to auxin or ferricyanide would activate proton release either by increasing redox-associated proton movement or by activating a proton-pumping ATPase. The redox system and the auxin-stimulated

proton release appear to be controlled by calmodulin, since calmodulin antagonists and the permeable calcium chelator TMB-8 inhibit both auxin-induced proton release and transmembrane electron transport both with cells and with NADH-loaded vesicles (Fig. 2).

Photocontrol of Plasma Membrane Redox and Proton Release

Light causes a remarkable stimulation of transplasma membrane electron transport and associated proton release. Effects are seen in both green (photosynthetic) and non-green tissue (Dharmawardhane *et al.*, 1987; Bown *et al.*, 1988; Elzenga and Prins, 1989; Revis and Misra, 1988).

The main effect of red light appears to result from photosynthesis since it is inhibited by DCMU. Production of sugar or other substrate for the transmembrane enzyme is the probable basis. It is not clear if the phytochrome system, which responds reciprocally to red vs. far red illumination, has any effect on the plasma membrane redox system (Wagner *et al.*, 1988), but changes in cytosolic redox state by this system could easily be reflected in plasma membrane electron transport activity.

There has been extensive study of blue light effects on the redox state of flavin and a cytochrome in plasma membranes (Briggs and Iino, 1983; Widell, 1987; Schmidt, 1984). Blue light also stimulates transplasma membrane electron transport in non-green tissue (Revis and Misra, 1988). It is logical to consider if the components reponsive to blue light are part of the transmembrane electron transport system (Møller and Crane, 1989; Dahse *et al.*, 1989). Blue light is involved in control of elongation growth and opening of stomata, and the effect has been attributed to the LIAC reaction involving reduction of a flavin-cytochrome b_{560} complex in the plasma membrane. If this redox change is connected to the transmembrane electron transport, it would be a basis for the H^+ release observed with blue light exposure (Raghavendra, 1990; Warpeha and Kaufman, 1990; Shimazaki *et al.*, 1986).

Conclusions

In both plant and animals cells, proton release accompanies electron transfer across the plasma membrane. In both, oxygen can play a role as a natural electron acceptor, but there appears to be an involvement of iron compounds, such as diferric transferrin and iron chelates in the soil, with the oxidoreductase system. Reduced pyridine nucleotides are the primary candidates for electron and proton donors, but ascorbate and glutathione

have not been excluded. Even though both plant and animal plasma membranes have transmembrane electron transport systems, it is not clear how much proton transfer is directly related to the electron transport system. The best evidence for such a relationship is in plants. On the other hand, in both there is good evidence that a large part of the proton transport associated with electron transport can be by activation of channels for proton movement, the Na^+/H^+ antiport in animals and the H^+-ATPase in plants. Only with a full knowledge of the components of the plasma membrane redox systems and analysis of their functions by molecular biological techniques and studies of isolated enzymes in vesicles will we get a clear picture of their function.

For the mechanism of channel activation by the redox system, two approaches stand out. One is generation of a local pH change to activate an allosteric site on the channel with protons. The other is the developing idea that oxidation–reduction of membrane components can activate protein kinases to phosphorylate control sites on channels. Examples of redox activation of protein kinase is seen in the effects of plastoquinone redox state on phosphorylation of LHC II protein in chloroplasts and the effect of ferricyanide on activation of tyrosine kinase in erythrocytes. Blue light redox also activates a protein kinase and causes phosphorylation of a specific membrane protein (Short and Briggs, 1990). Quinones may be a central feature of these kinase control systems.

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References

Anderson, J. M., and Andersson, B. (1988). *Trends Biochem. Sci* **13**, 351–355.
Askerlund, P. (1990). *Redox Processes of Plant Plasma Membrane*, Ph. D. Thesis, University of Lund, Sweden.
Askerlund, P., Larsson, C., and Widell, S. (1988). *FEBS Lett.* **239**, 23–28.
Barr, R. (1987). In *Redox Functions of the Eukaryotic Plasma Membrane* (Ramirez, J. M., ed.), Consejo Superior de Investigaciones Cientificas, Madrid, pp. 28–40.
Barr, R. (1988). *J. Bioenerg. Biomemb.* **23**, 443–467.
Barr, R., Craig, T. A., and Crane, F. L. (1985). *Biochim. Biophys. Acta* **812**, 49–54.
Barr, R., Martin, O., and Crane, F. L. (1987). *Proc. Indiana Acad. Sci.* **96**, 139–144.
Barr, R., Böttger, M., and Crane, F. L. (1990a). *Plant Physiol.* **93**, S,52.
Barr, R., Böttger, M., and Crane, F. L. (1990b). *Proc. Indiana Acad. Sci.* **98**, in press.
Barr, R., Brightman, A., Morré, D. J., and Crane, F. L. (1990c). *J. Cell. Biol.* **111**, 72a.
Bates, G. W., Workman, E. F., Jr. and Schlabach, M. R. (1973). *Biochem. Biophys. Res. Commun.* **50**, 84–90.

Bernstein, M., Dahse, I., Müller, E., Petzold, U. (1989). *Biochem. Physiol. Pflanzen.* **185**, 343–356.
Beyer, R. E., Nordenbrand, K., and Ernster, L. (1987). *Chem. Scr.* **27**, 145–153.
Bienfait, H. F. (1985). *J. Bioenerg. Biomembr.* **17**, 73–83.
Bienfait, H. F. (1988a). In *Plasma Membrane Oxidoreductases in Control of Animal and Plant Growth* (Crane, F. L., Morré, D. J., and Löw, H., eds.), Plenum Press, New York, pp. 89–93.
Bienfait, H. F. (1988b). *J. Plant. Nutr.* **11**, 605–629.
Bienfait, H. F., and Lüttge, U. (1988). *Plant Physiol. Biochem.* **26**, 1–7.
Boniface, J., and Reichert, L. (1990). *Science* **247**, 61–63.
Böttger, M., Crane, F. L., and Barr, R. (1991). *Oxidoreduction at the Plasma Membrane Vol. II* (Crane, F. L., Morré, D. J., and Low, H., eds.) CRC Press, Boca Raton, pp. 207–236.
Böttger, M. (1989). In *Plant Membrane Transport* (Dainty, J., De Michelis, M. I., Marré, E., and Rasi-Caldogno, F., eds.), Elsevier, Amsterdam, pp. 55–60.
Böttger, M., and Hilgendrof, F. (1988). *Plant Physiol.* **86**, 1038–1043.
Böttger, M., and Lüthen, H. (1986). *J. Exp. Bot.* **37**, 666–675.
Bottger, M., Soll, H.-J., and Bigdon, M. (1985). *Biol. Plant.* (*Praha*) **27**, 125–130.
Bown, A. W., and Crawford, L. A. (1988). *Plant. Physiol.* **73**, 170–174.
Briggs, W. R., and Iino, M. (1983). *Philos. Trans. R. Soc. London, Ser. B*, **303**, 347–359.
Brightman, O. A., Barr, R., Crane, F. L., and Morré, D. J. (1988) *Plant Physiol.* **86**, 1264–1269.
Bruggeman, W., Moog, P. R., Nakagawa, H., Janiesch, P., and Kuiper, P. J. C. (1990). *Physiol. Plant* **79**, 339–346.
Brummer, B., Felle, H., and Parish, R. W. (1984). *FEBS Lett.* **174**, 223–227.
Cakmak, I., van de Wetering, D. A. M., Marschner, H., and Bienfait, H. F. (1987). *Plant. Physiol.* **90**, 151–156.
Caubergs, R., Asard, H., and De Greef, J. A. (1988). In *Plasma Membrane Oxidoreductases in Control of Animal and Plant Growth* (Crane, F. L., Morré, D. J., and Löw, H., eds.), Plenum Press, New York, pp. 273–282.
Caughlin, S., and Hind, G. (1987). *J. Biol. Chem.* **262**, 8402–8406.
Chmelar, M., and Giacobino, J.-P. (1976). *Int. J. Biochem.* **7**, 159–163.
Clark, M. G., Patrick, E. J., Patten, G. S., Crane, F. L., Löw, H., and Grebing, C. (1981). *Biochem. J.* **200**, 565–572.
Cleland, R. E. (1976). *Plant. Physiol.* **58**, 210–213.
Cleland, R. E. (1987). In *Plant Hormones and Their Role in Plant Growth and Development* (Davies, P. J., ed.), Martinus Nijhoff Publishers, Dordrecht, pp. 132–148.
Cleland, R. E., and Rayle, D. L. (1978). *Bof. Mag. Spec. Issue* **1**, 125–139.
Cleland, R. E., Prins, H. B. A., Harper, J. R. and Higinbotham, N. (1977). *Plant Physiol.* **59**, 395–400.
Coleman, J. O. D., and Chalmers, J. D. C. (1988). In *Plasma Membrane Oxidoreductases in Control of Animal and Plant Growth* (Crane, F. L., Morré, D. J., and Löw, H., eds.), Plenum Press, New York, pp. 71–80.
Crane, F. L. (1990). In *Highlights in Ubiquinone Research* (Lenaz, G., Barnabei, O., Rabbi, A., and Battino, M., eds.), Taylor and Francis, London, pp. 3–20.
Crane, F. L., and Barr, R. (1989). *CRC Crit. Rev. Plant Sci.* **8**, 273–307.
Crane, F. L., Sun, I. L., Clark, M. G., Grebing, C., and Löw, H. (1985a). *Biochim. Biophys. Acta* **811**, 233–264.
Crane, F. L., Löw, H., and Clark, M. G. (1985b). In *The Enzymes of Biological Membranes*, Vol. 4 (Martonosi, A. N., ed.), Plenum Press, New York, pp. 465–510.
Crane, F. L., Morré, D. J., and Löw, H., eds. (1988a). In *Plasma Membrane Oxidoreductases in Control of Animal and Plant Growth*, (Crane, F. L., Morré, D. J., and Löw, H., eds.), Plenum Press, New York, pp. 1–443.
Crane, F. L., Barr, R., Morré, D. J., Brightman, A. O., and Craig, T. A. (1988b). In *Current Topics in Plant Biochemistry and Physiology*, Vol. 7 (Randall, D. D., Blevins, D. G., and Campbell, W. H., eds.), University of Missouri Press, Columbia, p. 224.
Crane, F. L., Morré, D. J., and Löw, H., eds. (1990a). *Oxidoreduction at the Plasma Membrane: Relation to Growth and Transport*, Vol. I, Animals, CRC Press, Boca Raton, p. 318.

Crane, F. L., Löw, H., Sun, I. L., Morré, D. J., and Faulk, W. P. (1990b). In *Growth Factors: From Genes to Clinical Applications* (Sara, V., Hall, K., and Löw, H., eds.), Raven Press, New York, p. 129–140.

Crane, F. L., Sun, I. L., Sun, E., and Morré, D. J. (1991a). In *Biomedical and Clinical Aspects of Coenzyme Q*, Vol. 6 (Folkers, K., and Littarru, G.-P., eds.), Elsevier, Amsterdam, 59–70.

Crane, F. L., Sun, I. L., Brightman, A., Morré, D. J., and Löw, H. (1990d). *J. Cell. Biol.* **111**, 231a.

Crane, F. L., Morré, D. J., and Löw, H., eds. (1991). *Oxidoreduction at the Plasma Membrane: Relation to Growth and Transport*, Vol. II, Plants, CRC Press, Boca Raton, 2928.

Dahse, I., Bernstein, M., Müller, E., and Petzold, U. (1989). *Biochem. Physiol. Pflanzen* **185**, 145–180.

Dharmawardhane, S., Stern, A. I., and Rubinstein, B. (1987). *Plant Sci.* **51**, 193–198.

Dilley, R. A., and Chiang, G. G. (1988). In *Plasma Membrane Oxidoreductases in Control of Animal and Plant Growth* (Crane, F. L., Morré, D. J., and Löw, H., eds.), Plenum Press, New York, pp. 199–208.

Dormandy, T. L., and Zarday, Z. (1965). *J. Physiol.* **180**, 684–707.

Elzenga, J. T. M., and Prins, H. B. A. (1987). *Plant Physiol.* **85**, 239–242.

Elzenga, J. T. M., and Prins, H. B. A. (1989). *Plant Physiol.* **91**, 68–72.

Elzenga, J. T. M., Staal, M., and Prins, H. B. A. (1989). *Physiol. Plant.* **76**, 379–385.

Faulk, W. P., Torry, D. S., Harats, H., McIntyre, J. A., and Taylor, C. (1988). In *Plasma Membrane Oxidoreductases in Control of Animal and Plant Growth* (Crane, F. L., Morré, D. J., and Löw, H., eds.), Plenum Press, New York, pp. 173–180.

Faulk, W. P., Harats, H., Crane, F., and Sun, I. L. (1989). In *9th International Conference on Proteins of Iron Transport*, Brisbane, Abstracts, p. 53.

Faulk, W. P., Harats, H., McIntyre, J. A., Berczi, A., Sun, I. L., and Crane, F. L. (1990). *Am. J. Reprod. Immunol.* **21**, 151–154.

Federico, R., and Giartosio, C. E. (1983). *Plant Physiol.* **73**, 182–184.

Fry, M. (1989). *Biochem. Biophys. Res. Commun.* **158**, 469–473.

Fuhrmann, G. F., Fehlan, R., Schneider, H., and Knauf, P. A. (1989). *Biochim. Biophys. Acta* **983**, 179–185.

Garcia-Cañero, R., Diaz-Gil, J. J., and Guerra, M. A. (1987). In *Redox Functions of the Eukaryotic Plasma Membrane* (Ramirez, J. M., ed.), Consejo Superior de Investigaciones Cientificas, Madrid, pp. 42–47.

Gayda, D. P., Crane, F. L., Morré, D. J., and Löw, H. (1977). *Proc. Indiana Acad. Sci.* **86**, 385–390.

Giannini, J. L., and Briskin, D. P. (1988). *Arch. Biochem. Biophys.* **260**, 653–660.

Goldbach, H. E., Hartmann, D., and Rötzer, T. (1990). *Physiol. Plant.* **80**, 114–118.

Goldenberg, H., Grebing, C., and Löw, H. (1983). *Biochem. Int.* **6**, 1–7.

Goldenberg, H., Eder, M., Pumm,, R., and Dodel, B. (1988). In *Plasma Membrane Oxidoreductases in Control of Animal and Plant Growth* (Crane, F. L., Morré, D. J., and Löw, H., eds.), Plenum Press, New York, pp. 131–152.

Golub, E. S., Diaz de Pagan, T., Sun, I., and Crane, F. L. (1988). In *Plasma Membrane Oxidoreductases in Control of Animal and Plant Growth* (Crane, F. L., Morré, D. J., and Löw, H., eds.), Plenum Press, New York, pp. 313–321.

Gopalakrishna, R., and Anderson, W. B. (1989). *Proc. Natl. Acad. Sci. USA* **86**, 6758–6762.

Grebing, C., Crane, F. L., Löw, H., and Hall, K. (1984). *J. Bioenerg. Biomembr.* **16**, 517–534.

Grinstein, S., Rotin, D., and Mason, M. J. (1989). *Biochim. Biophys. Acta* **988**, 73–97.

Gross, G. G., James, C., and Elstner, E. F. (1977). *Planta* **136**, 271–276.

Guern, J., Mathieu, Y., Ephritikhine, G., Ulbrich-Eberius, C. I., Lüttgi, U., Marré, M. T. and Marré, E. (1988). *Plasma Membrane Oxidoreductases in Control of Animal and Plant Growth* (Crane, F. L., Marré, D. J. and Lów, H. eds.). Plenum Press, New York, p. 412.

Hager, A., Menzel, H., and Krauss, A. (1971). *Planta* **100**, 47–75.

Hall, W., Wu, M., Crane, F. L., Takahashi, H., Tamura, S., and Folkers, K. (1966). *Biochem. Biophys. Commun.* **25**, 373–377.

Hassidim, M., Rubinstein, B., Lerner, H., and Reinhold, L. (1987). *Plant Physiol.* **85**, 872–875.

Henderson, L. M., Chappell, J. B., and Jones, O. T. G. (1988). *Biochem. J.* **251**, 563–568.

Heyworth, P. G., and Badwey, J. A. (1990). *J. Bioenerg. Biomembr.* **22**, 1–26.
Jones, G. J., and Morel, F. M. M. (1988). *Plant Physiol.* **87**, 143–147.
Jones, G. J., Palenik, B. P., and Morel, F. M. M. (1987). *J. Physiol.* **23**, 237–244.
Kalin, A., Norling, B., Appelkvist, E. L., and Dallner, G. (1987). *Biochim. Biophys. Acta* **926**, 70–78.
Kant, J. A., and Steck, T. L. (1972). *Nature (London)* **240**, 26–28.
Koshio, O., Akanuma, Y., and Kasuga, M. (1988). *Biochem. J.* **250**, 95–101.
Krüger, S., and Böttger, M. (1988). In *Plasma Membrane Oxidoreductases in Control of Animal and Plant Growth* (Crane, F. L., Morré, D. J., and Löw, H., eds.), Plenum Press, New York, pp. 105–114.
Ladoux, A., Cragoe, E. J., Jr., Gery, B., Arbita, J. P., and Frelin, C. (1987). *J. Biol. Chem.* **262**, 811–815.
Lenaz, G., ed. (1985). *Coenzyme Q*, Wiley, Chichester.
Leong, T.-Y., and Briggs, W. R. (1981). *Plant. Physiol.* **67**, 1042–1046.
Löbler, M., and Klämbt, D. (1985). *J. Biol. Chem.* **260**, 9854–9859.
Löppert, H. (1983). *Planta* **159**, 329–335.
Löw, H., and Crane, F. L. (1978). *Biochim. Biophys. Acta* **515**, 141–161.
Löw, H., Sun, I. L., Navas, P., Grebing, C., Crane, F. L., and Morré, D. J. (1986). *Biochem. Biophys. Res. Commun.* **139**, 1117–1123.
Löw, H., Grebing, C., Lindgren, A., Tally, M., Sun, I. L., and Crane, F. L. (1987). *J. Bioenerg. Biomembr.* **19**, 535–550.
Löw, H., Lindgren, A., Crane, F. L., Sun, I. L., Toole-Simms, W., and Morré, D. J. (1988). In *Plasma Membrane Oxidoreductases in Control of Animal and Plant Growth* (Crane, F. L., Morré, D. J., and Löw, H., eds.), Plenum Press, New York, pp. 153–152.
Löw, H., Crane, F. L., Morré, D. J., and Sun, I. L. (1990). In *Oxidoreduction at the Plasma Membrane: Relation to Growth and Transport* (Crane, F. L., Morré, D. J., and Löw, H., eds.), CRC Press, Boca Raton, pp. 29–66.
Low, P., Geahlin, R. L., Mehler, E., and Harrison, M. L. (1990). *Biomed. Biochim. Acta* **49**, 135–144.
Lucas, W. J., and Kochian, L. V. (1988). In *Plasma Membrane Oxidoreductases in Control of Animal and Plant Growth* (Crane, F. L., Morré, D. J., and Löw, H., eds.), Plenum Press, New York, pp. 219–232.
Lüthje, S., and Böttger, M. (1989). *Biochim. Biophys. Acta* **977**, 335–340.
Mäder, M., Meyer, Y., and Bopp, M. (1975). *Planta* **122**, 259–268.
Marré, E., Marré, M. T., Albergoni, F. G., Trockner, V., and Moroni, A. (1988). In *Plasma Membrane Oxidoreductases in Control of Animal and Plant Growth* (Crane, F. L., Morré, D. J., and Löw, H., eds.), Plenum Press, New York, pp. 233–242.
Marré, M. T., Moroni, A., Albergoni, F. G., and Marré, E. (1988). *Plant Physiol.* **87**, 25–29.
Mitchell, R. (1983). *Trends Biochem. Sci.* **8**, 117–119.
Møller, I. M., and Crane, F. L. (1990). In *The Plant Plasma Membrane* (Larsson, C., and Møller, I. M., eds.), Springer-Verlag, Berlin, pp. 93–126.
Møller, I. M., and Lin, W. (1986). *Annu. Rev. Plant. Physiol.* **37**, 309–334.
Morré, D. J., and Crane, F. L. (1990). In *Oxidoreduction at the Plasma Membrane: Relation to Growth and Transport*, Vol. 1, Animals (Crane, F. L., Morré, D. J., and Löw, H., eds.), CRC Press, Boca Raton, pp. 67–84.
Morré, D. J., Vigil, E. L., Frantz, C., Goldenberg, H., and Crane, F. L. (1978). *Eur. J. Cell. Biol.* **18**, 213.
Morré, D. J., Navas, P., Penel, C., and Castillo, F. J. (1986). *Protoplasma* **133**, 195–197.
Morré, D. J., Auderset, G., Penel, C., and Canut, H. (1987a). *Protoplasma* **140**, 133–140.
Morré, D. J., Navas, P., and Crane, F. L. (1987b). In *Redox Functions of the Eukaryotic Plasma Membrane* (Ramirez, J. M., ed.), Consejo Superior de Investigaciones Cientificas, Madrid, pp. 92–116.
Morré, D. J., Brightmann, A., Wang, J., Barr, R., and Crane, F. L. (1988a). In *Plasma Membrane Oxidoreductases in Control of Animal and Plant Growth* (Crane, F. L., Morré, D. J., and Löw, H., eds.), Plenum Press, New York, pp. 45–56.
Morré, D. J., Brightman, A. O., Wu, L.-Y., Barr, R., Leak, B., and Crane, F. L. (1988b). *Physiol. Plant.* **73**, 187–193.

Morré, D. J., Brightman, A. O., and Crane, F. L. (1991). In *Plant Signalling, Plasma Membrane, and Change of State* (ed. C. Penel and H. Treppin), University of Geneva, Geneva, Switzerland, p. 59–77.

Navas, P., Sun, I. L., Morré, D. J., and Crane, F. L., (1988). In *Plasma Membrane Oxidoreductases in Control of Animal and Plant Growth* (Crane, F. L., Morré, D. J., and Löw, H., eds.), Plenum Press, New York, p. 339.

Neufeld, E., and Bown, A. W. (1987). *Plant Physiol.* **83**, 895–899.

Norling, B., Glazek, E., Nelson, B. D., and Ernster, L. (1974). *Eur. J. Biochem.* **47**, 475–482.

Novak, V. A., and Ivankina, N. G. (1978). *Sov. Plant Physiol.* **25**, 315–321.

Peschek, G. A., Kurz, M. A., and Erber, W. W. A. (1988). *Physiol. Plant.* **73**, 175–181.

Polevoy, V. V., and Salamatova, T. (1977). In *Regulation of Cell Membrane Activities in Plants* (Marré, E., and Cifferi, O., eds.), Elsevier, Amsterdam, pp. 209–215.

Prins, H. B. A., and Elzenga, J. T. M. (1991). In *Oxidoreduction at the Plasma Membrane: Relation to Growth and Transport*, Vol. II, Plants (Crane, F. L., Morré, D. J., and Löw, H., eds.), CRC Press, Boca Raton, p. 149–166.

Qiu, Z.-S., Rubinstein, B., and Stern, A. I. (1985). *Planta* **165**, 383–391.

Raghavendra, A. S. (1990). *Plant, Cell, Environ.* **13**, 105–110.

Ramasarma, T., Swaroop, A., MacKellar, W., and Crane, F. L. (1981). *J. Bioenerg. Biomembr.* **13**, 241–253.

Ramirez, J. M., ed. (1987). *Redox Functions in the Eukaryotic Plasma Membrane*, Consejo Superior de Investigaciones Cientificas, Madrid.

Revis, S., and Misra, P. C. (1988). *Biochem. Physiol. Pflanzen* **183**, 487–494.

Robertson, R. N. (1991). In *Oxidoreduction at the Plasma Membrane: Relation to Growth and Transport*, Vol. II, Plants (Crane, F. L., Morré, D. J., and Löw, H., eds.), CRC Press, Boca Raton, p. 1–20.

Rubinstein, B., and Stern, A. I. (1986). *Plant Physiol.* **80**, 805–811.

Rubinstein, B., Stern, A. I., and Stout, B. (1984). *Plant Physiol.* **76**, 386–391.

Sardet, C., Counillon, L., Franchi, A., and Pouyssegur, J. (1990). *Science* **247**, 723–726.

Schmidt, W. (1984). *Bioscience* **34**, 698–704.

Serrano, R. (1985). *Plasma Membrane ATPase of Plants and Fungi*, CRC Press, Boca Raton.

Shimazaki, K., Iino, M., and Zeiger, E. (1986). *Nature* (*London*) **319**, 324–326.

Short, T. W., and Briggs, W. R. (1990). *Plant Physiol.* **92**, 179–185.

Sun, E. E., Sun, I. L., and Crane, F. L., (1990). *Proc. Indiana Acad. Sci.* **98**, in press.

Sun, I. L., and Crane, F. L. (1984). *Proc. Indiana Acad. Sci.* **93**, 267–274.

Sun, I. L., and Crane, F. L. (1985). *Biochem. Pharmacol.* **34**, 617–622.

Sun, I. L., and Crane, F. L. (1988). In *Plasma Membrane Oxidoreductases in Control of Animal and Plant Growth* (Crane, F. L., Morré, D. J., and Löw, H., eds.), Plenum Press, New York, pp. 181–190.

Sun, I. L., and Crane, F. L., (1990). In *Oxidoreduction at the Plasma Membrane: Relation to Growth and Transport*, Vol. I, Animals (Crane, F. L., Morré, D. J., and Löw, H., eds.), CRC Press, Boca Raton, pp. 257–280.

Sun, I. L., and Navas, P. (1987). In *Redox Functions of the Eukaryotic Plasma Membrane* (Ramirez, J. M., ed.), Consejo Superior de Investigaciones Cientificas, Madrid, pp. 65–89.

Sun, I. L., Crane, F. L., Grebing, C., and Löw, H. (1984). *J. Bioenerg. Biomembr.* **16**, 583–595.

Sun, I. L., Navas, P., Crane, F. L., Chou, J. Y., and Löw, H. (1986a). *J. Bioenerg. Biomembr.* **18**, 471–486.

Sun, I. L., Crane, F. L., and Chou, J. Y. (1986b). *Biochim. Biophys. Acta* **886**, 327–336.

Sun, I. L., Crane, F. L., Löw, H., and Grebing, C. (1987a). *Biochem. Biophys. Res. Commun.* **125**, 649–654.

Sun, I. L., Garcia-Cañero, R., Liu, W., Toole-Simms, W., Crane, F. L., Morré, D. J., and Löw, H. (1987b). *Biochem. Biophys. Res. Commun.* **145**, 467–473.

Sun, I. L., Navas, P., Crane, F. L., Morré, D. J., and Löw, H. (1987c). *J. Biol. Chem.* **262**, 15915–15921.

Sun, I. L., Toole-Simms, W., Crane, F. L., Morré, D. J., Löw, H., and Chou, J. Y. (1988a). *Biochim. Biophys. Acta* **938**, 17–23.

Sun, I. L., Toole-Simms, W., Crane, F. L., Morré, D. J., Löw, H., and Chou, J. Y. (1988b). *J. Bioenerg. Biomembr.* **20**, 383–391.

Sun, I. L., Sun, E. E., Crane, F. L., and Morré, D. J. (1990). *Biochem. Biophys. Res. Commun.* **172**, 979–984.

Sze, H. (1985). *Annu. Rev. Plant Physiol.* **36**, 175–208.

Theologis, A. (1989). In *Plant Biotechnology* (Kung, S.-D., and Arntzen, C. J., eds.), Butterworths, Boston, pp. 227–243.

Thorstensen, K., and Aisen, P. (1990). *Biochim. Biophys. Acta* **1052**, 29–35.

Toole-Simms, W. (1988). *Regulation of Proton Release from HeLa Cells by Ferric Reductase*, Ph. D. Thesis, Purdue University, West Lafayette, 160 pp.

Toole-Simms, W., Sun, I. L., Morré, D. J., and Crane, F. L. (1990). *Biochem. Int.* **21**, 761–769.

Trinder, D., Morgan, E. H., and Baker, E. (1988). *Biochim. Biophys. Acta* **943**, 440–446.

Tritsch, G. L., and Niswander, P. W. (1983). *Life Sci.* **32**, 1359–1364.

Trumpower, B. L., ed. (1982). *Function of Quinones in Energy Coupling Systems*, Academic Press, New York.

Ullrich, C. I., and Guern, J. (1990). *Planta* **180**, 390–399.

Ullrich, C. I., Kohler, K., Baier, M., Föster, B., and Hartung, W. (1990). *Bot. Acta* **103**, 214–221.

Venis, M. (1985). *Hormone Binding Sites in Plants.* Longman Group Ltd., Essex, England.

Waranimann, P., Sun, I. L., and Crane, F. L. (1986). *Proc. Indiana Acad. Sci.* **95**, 137–144.

Warpeha, K. M. F., and Kaufman, L. S. (1990). *Plant Physiol.* **92**, 495–499.

Weber, A., and Lüttge, U. (1988). *Z. Naturforsch.* **43c**, 257–263.

White, S., Teatle, R., Seligman, P. A., Rutherford, M., and Trowbridge, I. S. (1990). *Cancer Res.* **50**, 6295–6301.

Wikström, M. (1981). In *Mitochondria and Microsomes* (Lee, C. P., Schatz, G., and Dallner, G., eds.), Addison-Wesley, Reading, Massachusetts, pp. 249–269.

Wikström, M., and Krab, K. (1979). *Biochim. Biophys. Acta* **549**, 177–222.

Wikström, M., Krab, K., and Saraste, M. (1981). *Annu. Rev. Biochem.* **50**, 623–655.

Williams, L. T. (1989). *Science* **243**, 1564–1566.

Xia, J.-H., and Saglio, P. (1990). *Plant Physiol.* **93**, 453–459.

Yamamoto, Y., Niki, E., Eguchi, J., Kaminga, T., and Shimasak, H. (1985). *Biochim. Biophys. Acta* **819**, 29–36.

Zocchi, G., and Cocucci, S. (1990). *Plant Physiol.* **92**, 908–911.