Chapter- 3

Review of TransPMET System

3. REVIEW ON TRANSPLASMA MEMBRANE ELECTRON TRANSPORT SYSTEM [transPMET system]

3.1. 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 [172]. In most eukaryotic 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 [173]. 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 [174].

Other types of redox function are found in plasma membranes for special roles. The peroxide-generating NADPH oxidase in neutrophils used for killing bacteria [175], the xanthine oxidase that may have similar functions in addition to purine rescue [176], and the cytochrome b_5–dependent fatty acyl CoA desaturase [177] are examples. Proton transfer across the plasma membrane by activation of a channel has been associated with the neutrophil transmembrane NADPH oxidase [178]. 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 [179].

3.1.1. The evidence for trans PMET

Evidence for transplasma membrane electron transport is found in all animal cells examined, ranging from protozoa to man [180-183]. It is detected by reduction of impermeable dyes or complex ions by intact cells and by histochemistry [184, 185]. Electron transport reactions based on oxidation of NADPH by oxygen or the same impermeable compounds can be detected in isolated plasma membranes in nonvesicular form [186]. If the isolated membranes form sealed vesicles, then either the impermeable reductant or impermeable oxidant will not be available at its reaction site or the oxidoreductions observed would 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 [187]. The NADH in these vesicles can then be oxidized by external ferricyanide. Ascorbate has been inserted successfully by other methods [188, 189]. 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 [190]. If liver plasma membranes are not homogenized too vigorously, they show NADH dehydrogenase activity, which is consistent with the transmembrane electron transport. Erythrocyte membranes must be prepared in the open ghost form to show transmembrane electron transport [191].

Plasma membranes also have sites for NADH oxidation on their external surface [192]. 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 [193]. 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 [194]. It does not necessarily mean that NADH is the primary substrate since the NADH may reduce the primary substrate. For example, NADH may reduce semi dehydroascorbate to ascorbate, and ascorbate may be the primary electron donor to the transmembrane electron transport [195].

3.1.2. Properties 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 [196, 197]. 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 [198-201]. In isolated membranes the activity is up to 20 nmol per min per mg protein, unless diferric transferrin or hormones stimulate it. The measurement of the plasma membrane oxidase in cells is difficult because of the multifarious nature of cellular oxygen uptake. Since it is 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 1mM KCN and one–half of that oxygen uptake was inhibited by 0.2mM 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 [202]. 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 (P_{450}) 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 [199, 200]. Mammalian erythrocytes also have no transferrin receptors and differric transferrin does not activate NADH oxidase in these membranes [203]. However, if transferrin receptors are inserted into the erythrocyte membranes by *Falciparum* infection, then the cells show a transmembrane diferric transferrin reductase activity [204]. 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 [205], 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 [206, 207]. 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 [208]. 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 [207].

NADH diferric transferrin reductase activity can also be demonstrated using isolated liver membranes [186]. Three types of assay can be used to measure the activity.

(1) Direct measurement of a decrease of the diferric transferrin at 465nm 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 when diferric transferrin is added [186, 201]. 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 [204].

(3) Ferrous BPS is formed when diferric transferin 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 has a negative ξ 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 binding changes the conformation of the transferrin. If a transferrin receptor site is not important, then reduction of ferric desferrioximine would also be expected (–430mV) in the presence of BPS. This reduction is not seen with HeLa cells [202]. 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 [209], which means that reduction of diferric transferrin at that site is less energetically favoured 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 [201] 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 465nm under anaerobic conditions [186]. 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 [200]. 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 40μM [210]. 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 [211]. In other words, NADH oxidase stimulation and diferric transferrin reduction require 40μM 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. 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 [212].

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.

3.1.3. Components of the trans PMET

Plasma membranes have been reported to contain flavin, cytochromes of the b type, nonheme iron, coenzyme Q, α-tocopherol, thiol groups, and possibly copper [200, 213-215]. Coenzyme Q is the only component for which there is good evidence for participation in transmembrane electron transport.

3.1.4. Evidence for coenzyme Q function

Reduction of ferricyanide and diferric transferrin is inhibited by analogs of coenzyme Q, and the inhibition is reversed by addition of coenzyme Q [216, 217]. 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-hexa-decyl merca-

ptobenzoquinone are also good inhibitors of diferric transferrin reduction by cells [216]. 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) [218] procedure. Loss of activity is proportional to the amount of coenzyme Q removed [219].

A precedent for coenzyme Q function is transmembrane electron transport is seen in mitochondria [220, 221]. 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 [222]. Antimycin A and rotenone do not inhibit electron transport in plasma membranes [223, 224]. 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 [225].

3.1.5. Inhibitors of trans PMET

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 cellular 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 [226, 227], whereas adriamycin, *cis*-dichlorodiamine platinum II, actinomycin D, and bleomycin inhibit at low concentrations [228]. 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 system in cancer and infections by protozoa.

The important antitumor drugs, which are strong inhibitors of transplasmalemma electron transport, include adriamycin and related anthracyclines-bleomycin, *cis*-diaminodichloroplatinum II (cis-platin), actinomycin D, anthramycin, and retinoic acid [217, 228]. 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 [229]. Except for retinoic acid [230], proton release associated with the redox activity is also inhibited at the same concentrations starting at 10^{-7} M [228]. 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 [231-233]. The time is too short for the conjugate to release adriamycin to the nucleus.

3.1.6. Proton release associated with trans PMET: Animal cells

Transplasma membrane electron transport is associated with proton release from cells, as measured by a change in the external pH [234, 235]. 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 [202]. Since ferrocyanide and apotransferrin do not stimulate proton release, an electron acceptor is necessary. Inhibitors of transplasma membrane electron transport, such as adriamycin [203], bleomycin [236], cis-platin, and piericidin A, as well as monoclonal antibodies to the transferrin receptor [237], 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) [175] for mitochondria.

(b) the electron transport protein could act as a redox-controlled proton channel as proposed by Wikstrom [238], Wikstrom and Krab [239], and Wikstrom *et al.* [240], for cytochrome oxidase.

(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 [215].

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 portons released to ferricyanide reduced was around 2 or 3, which would be consistent with proton transfer through redox carriers during their oxidation-reduction cycle [235]. Later studies find 5 to 15 protons released per ferricyanide reduced, which would be more appropriate for activation of a channel [202]. Evidence that the Na^+/H^+ antiport could be the channel activated by ferricyanide was developed by Garcia-canero *et al.* [241], 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 [242]. Fuhrmann *et al.* [243] have also reported Na^+ influx into crythrocytes 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 trnasfer [237].

Diferric transferrin reduction is accompanied by a much greater proton release than with ferricyanide [174]. 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 trnsferrin 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 140nmol min^{-1} gww^{-1} for HeLa cells with 10μM diferric transferrin. The addition of 10μM apotransferrin to convert all iron to the tightly bound form decreases the reduction rate to 80nmol min^{-1} gww^{-1}. Further decrease may be achieved by incubating the ferric transferrin with apotransferrin before starting the reaction [210,244].

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 [245] 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 [237].

3.1.7. 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 [217, 228], 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 [210].

Retinoic acid inhibits transmembrane electron transport without inhibition of proton release [246]. Retinoic acid also stimulates proton release in the absence of oxidants or other agonists to activate the antiport, so the retinoic acid, which is permanent acid, may

activate the antiport by direct acidification of the allosteric site [247]. 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 [248].

The lack of retinoic acid inhibition of electron transport in SV40 trnasformed cells is further evidence that the portion of large T antigen inserted into the plasma membrane modified the electron – transport system [230].

3.1.8. Electron transport activate the antiport: The mechanism

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 [249].

(4) reduction of disulfide bonds which control antiport activity [243, 250].

The evidence that the antiport is regulated by phosphorylation on a serine [251] 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 has activated by low levels of H_2O_2 [252]. Low *et al.* [196] have shown that band 3 in erythrocytes (note that the erythrocyte antiport at 110kDa 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 [253]. Quinones, such as coenzyme Q, can generate H_2O_2 in membranes by autooxidation of semiquinones formed during the electron transport [220, 254]. Since there is now evidence that coenzyme Q functions in the plasma membrane electron transport [216, 219], and H_2O_2 generation occurs during NADH oxidation with isolated liver plasma membrane [255], 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 [196].

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 [256]

Addition to permeant acids to cells also activates the antiport [249]. 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 [194]. 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 [257].

The possible relation between the transmembrane electron transport system, the transferrin receptor, and the Na^+/H^+ antiport is diagrammed in [Fig.3.1]. Redox activation of a proton channel has also been described during the respiratory burst of erythrocytes, where rapid H_2O_2 formation occurs [167]. A role of protein kinase C in this process has also been indicated [258].

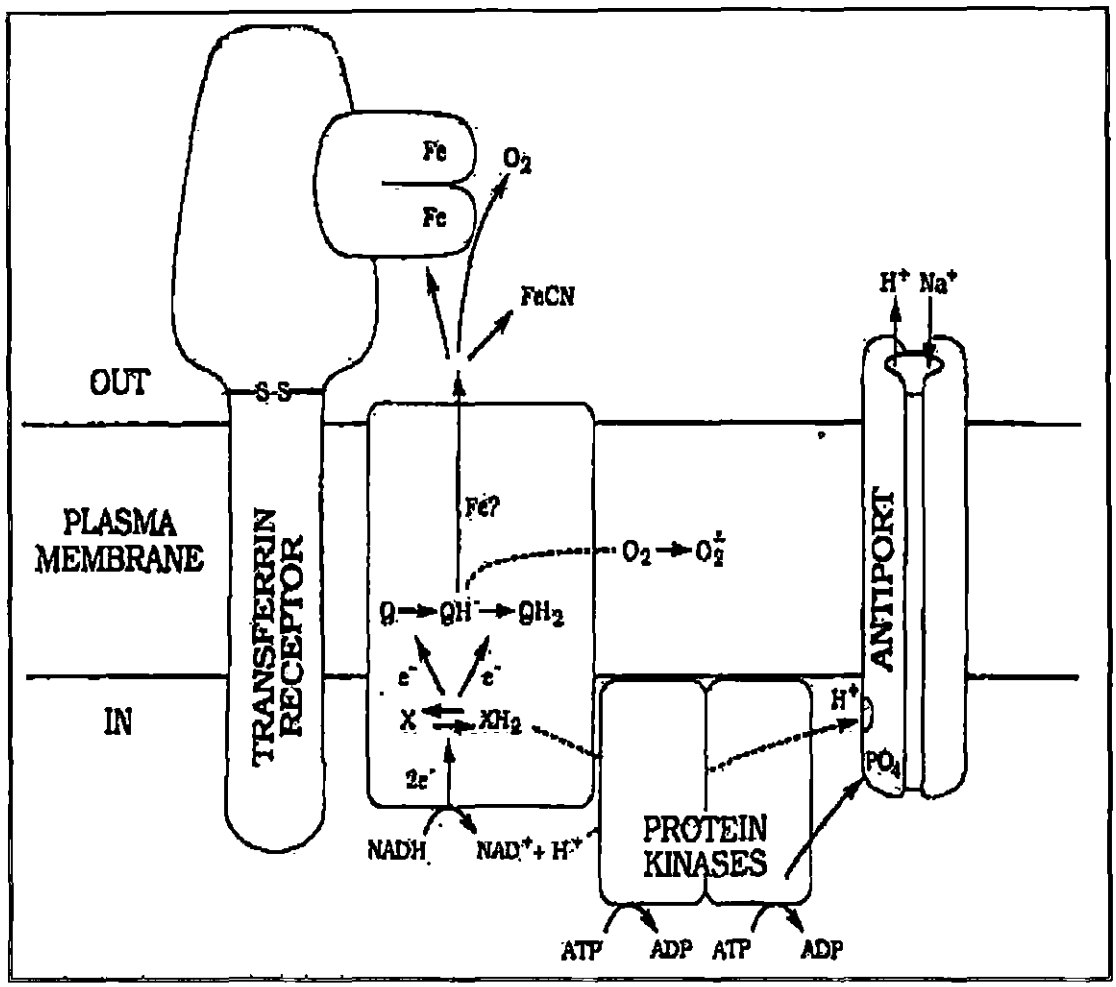

Fig.3.1. *Diagram showing the proposed relation between the transplasma membrane electron transport system, the transferrin receptor, and the Na^+/H^+ antiport for mammalian cells. Electron transport across the membrane is stimulated by ferric transferrin associated with the transferrin receptor. As 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 active 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 flavoprotein.*