Review article

Plasma membrane redox and control of sirtuin

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Abstract

We consider possible contributions of plasma membrane redox systems to aging control by sirtuin (SIR). Reported changes in plasma membrane redox introduced by calorie restriction (CR) may lead to activation of SIR. The most obvious effect would lie in the increase of NAD⁺ as a result of NADH oxidation. So the question arises, do the observed changes herald an increase in NADH oxidase under CR? The other possibility is an increase in expression of SIR by activation of plasma membrane oxidase. Previous experiments have shown that activation of the plasma membrane redox system can increase cellular NAD⁺ concentration. The plasma membrane redox systems are also involved in control of protein kinase activity through oxygen radical generation. This activity may be related to control of SIR expression.

The sirtuin family of NAD⁺ dependent protein deacetylases are important in the control of cellular functions related to aging and in promoting the long-term survival of cells (Cohen et al. 2004; Hekimi and Guarente 2003). These deacetylases require NAD⁺ as a cofactor for activity (Howitz et al. 2003). In mammals, nematodes and yeast calorie restriction (CR) delays aging by increasing the activity of the SIR groups of protein deacetylases (Weindruch and Sohal 1997; Lin et al. 2000; Nemoto and Finkel 2004). Control of SIR activity by CR can be based on several parameters. These include: (A) control of SIR expression, (B) control of NAD⁺ synthesis, (C) metabolic control of NAD⁺/NADH ratio or (D) control of nicotinamide concentration which is an inhibitor of SIR.

The mechanism for control of SIR activity may differ according to species. In yeast Anderson et al. (2003) found no increase in SIR expression under glucose deprivation (CR). On the other hand Anderson et al. (2003) did show an increase in nicotinamide deamidase with CR which would increase breakdown of

nicotinamide and prevent inhibition of SIR. Lin et al. (2002) suggest that the increase of aerobic respiration induced by CR will increase the NAD⁺/NADH ratio and CR increases the ratio of pyruvate to lactate in rat liver which supports an increase in the NAD⁺/NADH ratio (Diaz-Munoz et al. 2000). Of course the control mechanisms are not necessarily unrelated. Increase of NAD⁺ by respiration would increase production of its breakdown product nicotinamide which would have to be countered by an increase in the deamidase.

In rats CR increases expression of SIR and serum from CR rats increases expression and decreases aging in cell culture. CR also increases liver NAD⁺ as shown by decrease in lactate/pyruvate ratio (Diaz-Munoz et al. 2000). On the other hand, CR does not appear to produce a long term decrease in respiration (McCarter and McGee 1989) even though it may decrease mitochondrial electron transport (de Grey 2001). The role of the plasma membrane electron transport (PMOR) to keep NAD⁺ oxidized even if mitochondrial electron transport is inhibited by CR

has been discussed by de Grey (2001). Increased NADH in the mitochondria could be oxidized through reversal of the malate/aspartate shuttle to produce cytosolic NADH which can be oxidized by the plasma membrane electron transport. The oxidation of cellular NADH by the plasma membrane electron transport using external ferricyanide or iron transferrin as electron acceptors has been demonstrated (Navas et al. 1986; Merker et al. 2002). A high concentration of cytosolic NAD⁺ could be maintained by this sequence.

Control of NAD⁺

The objective of this discussion is to consider how the plasma membrane electron transport can contribute to the activation or expression of SIR under CR conditions. First, we consider the currently known components of transplasma membrane redox systems. Two NADH dehydrogenases have been implicated: (A) NADH cytochrome *b*₅ reductase (Navarro et al. 1995) and (B) voltage dependent anion channel protein (VDAC). (Baker et al. 2004). Two NADH/NADPH dehydrogenases are also recognized: (A) DT diaphorase (NQO1¹⁶) and (B) the cytochrome *b*₅₅₈ reductase which is related to the neutrophil P22^{phox} peroxide generating oxidase (Hoidal et al. 2003). The latter is mostly found in transformed cells or endothelial cells (Lambeth 2002). The other dehydrogenases are more generally distributed in mammalian cells. Further transfer of electrons across the plasma membrane appears to require coenzyme Q and an iron site on the exterior (Sun et al. 1992; Alcain et al. 1995). The cytochrome *b*₅ reductase, DT diaphorase, and VDAC can all use coenzyme Q as electron acceptor, whereas the cytochrome *b*₅₅₈ system (P22^{phox}) does not require coenzyme Q (Lambeth 2002). Reaction of oxygen can be based on the reduced coenzyme Q oxidase (Kishi et al. 1999) or probably on diferric transferrin (as discussed later). The cytochrome *b*₅₅₈ is a low potential cytochrome which reacts directly with oxygen. This NAD(P)H oxidase activity is found in five systems referred to as NOX-1 to NOX-5 (Lambeth 2002). They should not be confused with the reduced coenzyme Q oxidase, which has been designated as CNOX (Morre 1998). An external iron site on the plasma membrane has also been recognized (Alcain et al. 1994); it remains to be seen how it functions in the NADH oxidase systems.

An alternative catalyst for NADH oxidation of the plasma membrane is diferric transferrin. The stimulation of cellular NADH oxidation to form NAD⁺ has been directly demonstrated by measuring changes in NADH and NAD⁺ in cell culture when diferric transferrin is added to the cells (Navas et al. 1986; Merker et al. 2002). Forty-eight hour incubation of HeLa cells with diferric transferrin increased NAD⁺ levels by 45% (Navas et al. 1988). The function of diferric transferrin as a terminal oxidase at the plasma membrane has been obscured by attention to the role of transferrin in iron uptake and requires some explanation. Two methods have been used to show iron reduction in transferrin by NADH at the plasma membrane (Sun et al. 1987a). (A) The reduction of diferric transferrin at 465 nm is measured with plasma membrane and NADH under argon. No reduction occurs in air and introduction of air to the anaerobic cuvette causes immediate reoxidation of the bleached transferrin (Bates et al. 1973). This is a clear example of the oxidase function of transferrin. The rapid reoxidation also shows that reduction at the plasma membrane under air at neutral pH would not facilitate the uptake of ferrous iron by the cell. (B) The second assay involved the formation of ferrous bathophenanthroline disulfonate (BPS) with NADH and plasma membrane as measured by absorbance increase at 535 nm (Low et al. 1987). This assay was properly objected to as not pertinent to iron uptake since the presence of the ferrous chelator BPS altered the redox poise of the system to favor reduction of iron (Thorstensen and Romslo 1990). Therefore, this modified system cannot give evidence for the formation of ferrous iron for iron uptake. It does, however, show that electrons from NADH can be transferred by the plasma membrane to the iron in transferrin. In other words, BPS creates a condition similar to anaerobiosis to preserve the ferrous iron formed from the transferrin. A third assay measured the oxidation of NADH by absorbance at 340 nm in the presence of plasma membrane and diferric transferrin (Sun et al. 1987a, b). This assay shows that diferric transferrin increases the rate of NADH oxidation over the basal rate in the unsupplemented plasma membrane. This is consistent with either a transferrin stimulation of endogenous oxidase or catalysis of oxidation by redox turnover of iron in the ferric transferrin. It should be noted that this stimulation is maximum in plasma membrane with transferrin receptors (Sun et al. 1987a). Diferric lac-

toferrin also stimulates NADH oxidase with rat liver plasma membranes even though it is not involved in iron uptake (Sun et al. 1991). The diferric transferrin reductase activity as measured by these assays in rat liver plasma membrane is shown in Table 1.

A further complication to interpretation of the role of diferric transferrin in the plasma membrane oxidase is the presence of loosely bound iron (ascorbate reducible) on the diferric transferrin (Low et al. 1988). This iron would not be involved in the direct spectrophotometric analysis of diferric transferrin reduction at 465 nm. The excess iron may increase the rate of NADH oxidation measured at 340 nm since apotransferrin which would remove the excess iron also partially inhibits the oxidase (Low et al. 1987) (cf Table 1). NADH oxidation is still observed at a decreased rate after removal of excess iron (Sun et al. 1991; Brightman et al. 1992). The contribution of excess iron to the stimulation of the oxidase needs further study and may be important in understanding the CR response (Jordan and Kaplan 1994). The partial inhibition of NADH oxidase stimulation by apotransferrin may indicate a role for excess iron on the transferrin which can be removed by the apotransferrin (Sun et al. 1987a; Low et al. 1986). The effect of apotransferrin is seen under the NADH oxidase assay (change in absorbance at 340 nm) in Table 1. Note that the basic oxidase rate

of 5.6 is increased to 8.1 even in the presence of apotransferrin to remove any loosely bound iron. Inhibition of the NADH oxidase stimulation (but not the endogenous oxidase) by anti transferrin antibody indicates that the low affinity iron cannot function independent of the transferrin (Table 1).

It has been shown that CR can affect some of the components of the plasma membrane redox system. The ratio of coenzyme Q₉ and Q₁₀ can be maintained with age and dehydrogenase activity, such as DT diaphorase or semidehydro-ascorbate reductase can be increased (de Cabo et al. 2004). CR in humans may increase serum transferrin (Walford et al. 2002). How these changes affect the plasma membrane NADH or NADPH oxidase is not known. If the transferrin increase is confirmed, then a stimulation of cytosolic NADH oxidation can be expected (Navas et al. 1986; Brightman et al. 1992). If there is an increase in serum iron to overload the diferric transferrin, then a further stimulation of the diferric transferrin activation can be expected.

Other factors have been identified which can affect the PMOR. GTP increases the diferric transferrin stimulated NADH oxidase of liver plasma membrane (Moore et al. 1993). Control of PMOR by ras mutation (Crowe et al. 1993), calcium and cyclic AMP (del Castillo-Olivares et al. 1995; Rodriguez-Aguilera et al. 1993) have also been described. Any effects of CR on G proteins or adenylate cyclase could have an effect on cytosolic NAD⁺ through the PMOR. Triiodothyronine (T3) is decreased by CR (Walford et al. 2002) but it stimulates the endogenous PMOR NADH oxidase (Gayda et al. 1977). T3 effect on transferrin stimulation of the oxidase is not known. Since T3 inhibits NADH indophenol reductase in plasma membrane, we do not know what it will do to diferric transferrin reduction or stimulation of NADH oxidation by diferric transferrin.

The redox systems in the plasma membrane are in a position to directly control the concentration of cytosolic NAD⁺ which is needed for the activation of SIR. There are indications that CR can modify these systems, but much is still unknown about how they work.

Control of SIR expression

The redox systems in the plasma membrane have been shown to be involved in the control of gene

Table 1. Assay for diferric transferrin reduction by NADH in rat liver plasma membrane.

Additions and conditions	Rate of diferric transferrin reduction according to method of assay		
	Neq/min ⁻¹ /mg protein ⁻¹		
	465	340	535
Air, NADH, FeTf	0	14.7	—
Argon, NADH, FeTf	3.7	—	—
Air, No FeTf, NADH	0	5.6	0
Air, NADH, FeTf, BPS	—	—	7.0
Air, NADH, FeTf, ApoTf	—	8.1	—
Air, NADH, FeTf, FeTf ab	—	5.6	—

Assays 465 direct assay of diferric transferrin reduction by decrease in absorbance at 465 nm, 340 oxidation of NADH increased by diferric transferrin, 535 formation of ferrous bathophenanthroline disulfonate BPS.

Data calculated from Sun et al. (1987a); see also Sun et al. (1991, 1992); Brightman et al. (1992).

FeTf diferric transferrin, Apo Tf, apotransferrin.

FeTf ab transferrin antibody.

expression (Suzuki and Griendling 2003; Hoidal et al. 2003; Herkert et al. 2004; Rhee et al. 2003). Most attention has been focused on the action of the NOX 1-5 NADPH oxidases. These oxidases can be stimulated by hormones and growth factors to produce H_2O_2 . The H_2O_2 acts as a messenger to activate protein kinases or inactivate phosphotyrosine protein phosphatases (Lambeth 2002; Rhee et al. 2003). The NOX 1-5 NADPH oxidases have mostly been associated with gene activation in transformed, endothelial, or other specialized cells. It is not clear if they are significant in other normal tissue, such as liver or adipocytes. For example, NADPH dehydrogenase activity in liver plasma membrane is one half the activity of NADH dehydrogenase (Sun et al. 1987a; Brightman et al. 1992).

There is some evidence that the NADH oxidase system in the plasma membrane can also produce H_2O_2 . The CNOX or reduced coenzyme Q oxidase forms superoxide which can be converted to H_2O_2 (Berridge and Tan 2000). We do not have evidence that the diferric transferrin stimulation of the oxidase produces H_2O_2 , but that would be logical based on one electron transfer from the ferrous iron to oxygen (Bates et al. 1973). The iron site on the exterior of the membrane involved in ferricyanide reduction (Alcain et al. 1995; Walford et al. 2002) can also be a site for superoxide formation. Since CR causes modification in the components of the plasma membrane electron transport system (de Cabo et al. 2004) the change in activity may be a basis for induction of SIR. The induction may not be in response to H_2O_2 generation since activation of PMOR with ferricyanide as an external electron acceptor results in a transient expression of *c myc* and *c fos* (Wenner and Cutry 1990) in cultured cells and ferricyanide reduction would not be compatible with superoxide formation. The different components of the PMOR may support different mechanisms of message transduction. The activation of proton release from the cell by activation of PMOR by transferrin (Crane et al. 1991) to alter internal pH may also be related to the effects of CR (de Grey 2001).

Conclusions

There are several plasma membrane redox (PMOR) systems for oxidation of cytosolic NADH and

NADPH. Recognized components for NADH oxidation include NADH cytochrome b5 reductase (Villalba et al. 1997), NOQ-1 diaphorase (de Cabo et al. 2004), VDAC-1 (Baker et al. 2004), coenzyme Q oxidase (Kishi et al. 1999), an iron component (Alcain et al. 1995), and diferric transferrin (Sun et al. 1987b). There is evidence that CR can control some of these components (De Cabo et al. 2004). In addition to NOQ-1, the oxidation of NADPH in some cells is based on the NOX 1-5 GP22 type oxidases (Hoidal et al. 2003). It is not known if these enzymes can be controlled by CR or if there are transhydrogenase systems which can allow oxidation of NADH through NADP.

The evidence that NAD^+ levels in the cell can be controlled by the PMOR is evidence that CR effects could be mediated through PMOR. A study of NADH oxidase activity response to CR is needed. The activity of the plasma membrane NAD(P)H oxidase in liver cells can be directly measured with isolated liver cells. Of the oxygen uptake, 10 to 25% is insensitive to cyanide. Of the cyanide insensitive oxidase 60% is inhibited by the external electron acceptor ferricyanide which would displace oxygen as external electron acceptor. It would be of interest if CR would change the rate of cyanide insensitive but ferricyanide inhibited oxygen uptake of liver cells (Crane and Low 2001).

Control of gene expression by oxygen radical production through the NOX 1-5 enzyme activity is recognized but CR effects on the NOX enzyme are unknown. As an alternative, little is known about oxygen radical production by NADH oxidation and control by CR.

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