Transplasmalemma Electron Transport Is Changed in Simian Virus 40 Transformed Liver Cells

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Abstract

Transplasma membrane electron transport activity by fetal rat liver cells (RLA209-15) infected with a temperature-sensitive strain of SV40 has been measured with cells grown at the restrictive temperature (40°C) and permissive temperature (33°C). The transformed cells grown at 33°C had only one-half the rate of external ferricyanide reduction as the nontransformed cells held at 40°C. Both the $K_m$ and $V_{max}$ for ferricyanide reduction were changed in the transformed state. The change in $V_{max}$ can be based on a decrease of NADH in the transformed cells. The change in rate with ferricyanide does not depend on change in surface charge. Reduction of external ferricyanide was accompanied by release of protons from the cells. The ratio of protons released to ferricyanide reduced was higher in the transformed cells than in the non-transformed cells. Since the transplasma membrane electron transport has been shown to stimulate cell growth under limiting serum, the changes in the plasma membrane electron transport and proton release in transformed cells may relate to modification of growth control.

Key Words: Plasma membrane; pyridine nucleotide oxidation; temperature sensitive SV40; liver cells; transmembrane electron transport; cell transformation; enzyme kinetics.

Introduction

A transplasma membrane redox system, found in many types of cells, has been related to the control of cell growth. Activation of transplasma membrane electron transport with ferricyanide stimulates the growth of melanoma cells in serum-deficient media (Ellem and Kay, 1983) and promotes HeLa cell proliferation in the absence of fetal calf serum or other
growth factors (Sun et al., 1984a). A series of impermeable oxidants with redox potentials down to $-125\text{ mV}$ have similar growth promoting effects (Sun et al., 1984b). All of these oxidants are reduced by the transplasma membrane electron transport system. Oxidants which are not reduced by the transmembrane electron transport do not stimulate growth (Sun et al., 1984b). Growth-promoting hormones, such as insulin, stimulate growth in the absence of serum and also stimulate transmembrane redox activities (Sun et al., 1984a). Diferric transferrin, which is an essential growth factor for many cells, can act as an electron acceptor for the plasma membrane redox system (Crane et al., 1985a).

Redox effects on growth are not unexpected, since animal cells which have nonfunctional mitochondria still require oxygen for growth (Scheffer et al., 1981). Antineoplastic drugs (Sun and Crane, 1981, 1984a, b, c) which inhibit cell growth also inhibit the transplasma membrane redox system. There is also some evidence that virus-transformed or tumor cells have lower transmembrane redox activities than nontransformed cells. These cells include Simian Virus 40 transformed RLA209-15 liver cells (Sun et al., 1983), SV40-transformed RPNA209-1 pineal cells (I. L. Sun, unpublished), 3T3 cells (Crane et al., 1985b), Esb (LS178Y-ES and EbLS178Y-E) cells (Cherry et al., 1981), and hepatoma Mca-RH7777 cells (Crane et al., 1985b). A lower NADH ferricyanide reductase activity in purified plasma membrane from transformed 3T3 cells has been reported (Sheinin and Onodera, 1972). The transmembrane redox activity in transformed cells is also more sensitive to inhibition by antitumor drugs (Sun et al., 1983).

To understand the regulation of transmembrane redox systems in cancer cells it is necessary to have a valid control system in which to study the normal redox function. Cultured rat fetal liver cells (RLA209-15) that retain differentiated hepatic functions have been established by transforming normal liver cells with tsA mutant of SV40 that is temperature sensitive in the gene required for the maintenance of transformation (Chou and Schlegel-Haueter, 1981). The cell line expresses the transformed phenotype at the permissive temperature (33°C) but mimics the normal nontransformed hepatocytes at the restrictive temperature (40°C). This cell line provides us a favorable model for the study of the nature of transmembrane redox modification under reversible conditions of malignant transformation.

Numerous studies have shown that pyridine nucleotide pools are significantly altered in proliferating tissues in comparison to those of nonproliferating tissues (Jedeikin and Weinhouse, 1955; Caiger et al., 1962; Nemeth and Dickerman, 1960; Briggs, 1960; Burch and Von Dippe, 1964; Clark et al., 1966; Ferris and Clark, 1972). We have previously observed a decrease in NAD(H) levels of rat liver following administration of a carcinogen (2-acetylaminofluorene) (Sun et al., 1985). Furthermore, changes in pyridine