MITOCHONDRIAL AUTONOMY: DEPRESSED PROTEIN AND GLYCOPROTEIN SYNTHESIS IN MITOCHONDRIA OF SV-3T3 CELLS*

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1. Introduction

Mitochondria have been shown to possess essential and unique DNA [1], RNA [2, 3], and a complete system for the synthesis of protein [4, 5], glycoprotein [6–8], and glycolipid [9]. Several lines of evidence have raised the question of whether normal mitochondrial genetic processes might be altered in neoplastic cells. Levine [10] found that during transformation of cells by the oncogenic virus SV-40, the synthesis of mDNA is accelerated, as is that of nuclear DNA. Kara et al. [11] reported that RSV particles can replicate within mitochondria, illustrating the feasibility of the entrance and maintenance of viral information in the mitochondrion. Of particular interest is the recent demonstration that RNA of Venezuelan equine encephalomyelitis virus penetrated into isolated rat liver mitochondria [12], that replication of the virus occurred within the mitochondria [13], and that upon infection, the mitochondria switched to production of virus specified products as opposed to mitochondrion specified products [14]. Finally, mitochondrial membrane preparations of normal and hepatoma cells have been shown to differ by polyacrylamide electrophoresis, suggesting different products of mitochondrial protein synthesis [15]. These data prompted us to examine protein and glycoprotein synthesis by mitochondria of a contact inhibited mouse fibroblast (3T3) and its oncogenic virus transformed derivative, SV-40 3T3.

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2. Materials and methods

Mitochondria were prepared from trypsin harvests of 3T3 and SV-3T3 monolayers just prior to confluence. Cells were washed in 0.25 M sucrose and disrupted by a tight fitting Dounce homogenizer in 0.15 M sucrose; the homogenate was made 0.25 M in sucrose and submitted to differential centrifugation. Nuclei and debris were removed by 700 *g* for 10 min. A 5500 *g* mitochondrial pellet was washed, resuspended, and recentrifuged for a total of 5 washes. This final mitochondrial pellet was used for protein and glycoprotein synthesis experiments.

Membrane marker enzymes were assayed at various stages in the mitochondrial isolation procedure to determine purity of the preparation. Samples were homogenized in 0.1% Triton X-100 and then assayed for succinic dehydrogenase [16] (EC 1.3.99.1)–a mitochondrial marker; 5′-nucleotidase [17] (EC 3.1.3.5)–a plasma membrane marker; UDPase [18] (EC 3.1.3.6)–a smooth endoplasmic reticulum enzyme; and acid phosphatase [19] (EC 3.1.3.2)–a lysosomal marker enzyme. The data in table 1 illustrate that the five-times washed mitochondrial pellet was enriched in SDH, essentially free of UDPase and 5′-necleotidase, and slightly contaminated with acid phosphatase.

The purity of the preparation was examined by the effects of 100 μg/ml cycloheximide or chloramphenicol and 500 μg/ml RNAase on the incorporation of [^{14}C]leucine. Cycloheximide is known to inhibit protein synthesis on cytoplasmic ribosomes while chloramphenicol inhibits protein synthesis on mitochondrial ribosomes at these concentrations [20]. RNAase does not pass through the mitochondrial membrane

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Table 1
Activity of membrane marker enzymes in cell fractions (μmoles of product formed/mg protein/hr).

Fraction	3T3				SV-3T3			
	Succinic dehydrogenase	5′-Nucleotidase	UDPase	Acid phosphatase	Succinic dehydrogenase	5′-Nucleotidase	UDPase	Acid phosphatase
Unbroken, trypsin-harvested cells	78	2.4	7.2	0.014	56	2.0	6.8	0.011
1000 *g* pellet (nuclei and unbroken cells)	65	2.1	5.9	0.005	48	2.2	4.1	0.002
First 5500 *g* pellet (mitochondria 1)	49	1.1	2.6	0.031	35	1.7	1.7	0.029
Once washed pellet (mitochondria 2)	67	0.09	1.1	0.031	39	0.22	0.08	0.032
Three times washed pellet (mitochondria 4)	104	0*	0.2	0.010	87	0	0	0.006
Five times washed pellet (mitochondria 6)	156	0	0	0.006	112	0	0	0.004

* No detectable activity.

and thus inhibits only extramitochondrial protein synthesis [21]. Neither cycloheximide nor RNAase altered the amount of leucine incorporated by the 3T3 or SV-3T3 mitochondrial preparation while chloramphenicol inhibited incorporation 80–90% (fig. 1). These enzymic and incorporation data indicate that the cell fraction used in these experiments is mitochondrial and is free from significant microsomal contamination.

3. Results and discussion

There was a consistent difference in amount of leucine, glucose, and mannose incorporated into acid insoluble material by 3T3 and SV-40 3T3 mitochondria (fig. 2). In each instance incorporation by mitochondria from the 3T3 cells was greater than the incorporation by mitochondria from the SV-40 3T3 cells. SV-40 3T3 mitochondria incorporated only 60% as much leucine, 80% as much glucose, and 50% as much mannose as did 3T3 mitochondria per mg of mitochondrial protein. Thus both protein and glycoprotein synthesis is decreased in mitochondria by SV-40 oncogenic transformation of the cell.

The basis for this differential incorporation has not yet been determined. The different level of protein

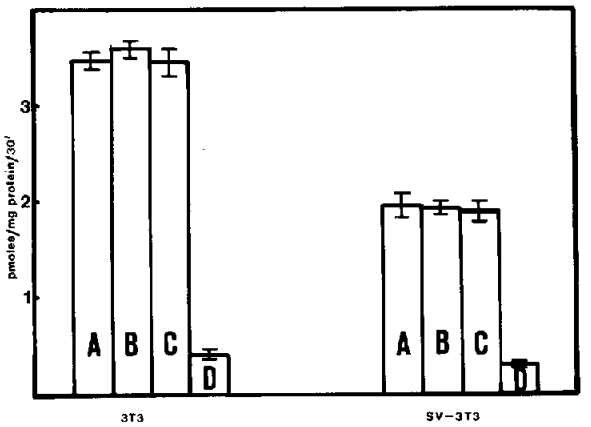

Fig. 1. Leucine incorporation by isolated mitochondria. Data represent pmoles of [^{14}C]leucine incorporated into acid and ethanol–ether (2:1, v:v) insoluble material per mg mitochondrial protein per 30 min. Incubation medium contained 5 mM sodium phosphate buffer, 50 mM Tris, 150 mM KCl, 10 mM $MgCl_2$, 2 mM EDTA, 10 mM ATP, 5 mM phosphoenolpyruvate 10 μg/ml pyruvic kinase, 22.5 μg/ml complete amino acid mixture ± leucine, 1–2 mg/ml mitochondrial protein, and 0.1 μCi [^{14}C]leucine, 0.05 μCi UDP-[^{14}C]glucose or 0.03 μCi GDP-[^{14}C]mannose, pH 7.6, as given previously [7]. Experiments added to the control (A) system either 500 μg/ml RNAase (B), 100 μg/ml cycloheximide (C), or 100 μg/ml chloramphenicol (D). Bars represent means ± S.D., n = 4.

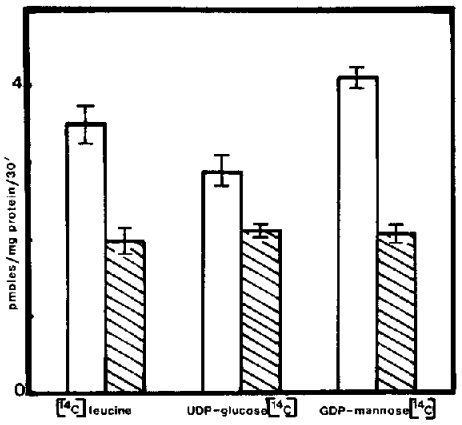

Fig. 2. Leucine, glucose and mannose incorporation by mitochondria isolated from 3T3 and SV-40 3T3 cells. Data represents pmoles incorporated per mg mitochondrial protein per 30 min. Open columns represent 3T3 mitochondria and cross-hatched columns represent SV-40 3T3 mitochondria. Bars represent means ± S.D., n = 8 mitochondrial preparations.

synthesis could be due to altered genetic messages (qualitatively or quantitatively different) delivered to the mitochondrial ribosome or to an altered state of readiness for synthesizing protein from the messages. The differential in glycoprotein synthesis could be due to the presence of altered mitochondrial acceptor molecules, to altered levels of mitochondrial glycosyl transferases, or to altered precursor pool size.

Growth rate of the cell might determine protein synthesis rates. It has been shown that mitochondrial protein synthesis is increased over adult rat liver rates in fetal liver and regenerating liver. If growth and division rate of the 3T3 cells were responsible for this mitochondrial protein synthesis differential, SV-3T3 incorporation would be expected to be higher not lower than that of 3T3 mitochondria since they continue to grow and divide after confluency is reached while 3T3 do not.

These data suggest that the mitochondria, along with the nucleus and the plasma membrane, may be a possible locus of oncogenic transformation. In the light of recent work linking viral replication and the mitochondrion [13–14], the mitochondrion may indeed play an important role in viral carcinogenesis.

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