Mitochondrial Autonomy: Incorporation of Monosaccharides into Glycoprotein by Isolated Mitochondria

Abstract. *Isolated intact mitochondria selectively incorporate monosaccharides from nucleotide diphosphate monosaccharides into protein. Fucose, mannose, glucose, and galactose were incorporated by the mitochondria into glycoprotein; xylose was not. Structural integrity of the mitochondria was not necessary for the incorporation of monosaccharide into glycoprotein; mitochondria broken by homogenization also incorporated monosaccharide. The monosaccharides incorporated into glycoprotein were localized in the inner mitochondrial membranes, the same membranes which contain the protein into which leucine is incorporated by the isolated mitochondria.*

The autonomy of the mitochondria has been demonstrated by reports indicating that mitochondria contain systems for the independent biosynthesis of various macromolecules of both structural and informational nature.

Mitochondria contain DNA (*1, 2*), ribosomes (*3*), RNA polymerase (*1*), transfer RNA (*4*), and amino acid activating enzymes (*5*) and are independently capable of synthesizing protein in vitro (*6–9*) and in vivo (*10*). It is the insoluble "structural proteins" of the inner membrane of the mitochondria which are in the main synthesized by the mitochondria (*6, 7*). Sialic acid, found in mitochondria as well as in other membrane systems, presumably is attached to protein (*11*), and mitochondria contain other glycoproteins (*12, 13*). Inasmuch as the structural proteins of the membrane are synthesized by the mitochondria and glycoproteins occur in the membranes of the mitochondria, we undertook the study of whether the isolated mitochondrion contains the enzymes necessary for the independent biosynthesis of glycoproteins.

Rat liver mitochondria were prepared from rats (250 g) which had been starved for 16 hours (*14*); the homogenates were washed five times with 0.25*M* sucrose and then centrifugated at 5000*g* for 10 minutes because the wash supernatant was not clear until the fourth wash. All glassware, instruments, and capped tubes were autoclaved; solutions and media were sterilized either by Millipore filtration (0.22 μ pore width) or by autoclaving. Bacterial contamination was measured by counting the viable organisms at the surface; samples (0.2 ml) were plated on blood agar or Sabouraud dextrose agar plates, incubated at 30°C, and observed daily. Our assay tubes after incubation contained less than 40 bacteria per milligram of mitochondrial protein.

Intact mitochondria incorporated leucine-^{14}C, glucose-^{14}C, galactose-^{14}C, mannose-^{14}C, and fucose-^{14}C into protein (Table 1). The nucleotide diphosphate monosaccharide-^{14}C was the substrate in each instance; xylose-^{14}C was not incorporated. Ribonuclease (final concentration 0.5 mg/ml) did not affect the incorporation of leucine into protein, a confirmation of earlier findings (*10*), and had no effect on the incorporation of monosaccharides. A ten-

Table 1. Incorporation of leucine-^{14}C and monosaccharide-^{14}C into protein and glycoprotein by isolated mitochondria. Data are expressed as counts per minute per milligram of mitochondrial protein. In each instance the mitochondria, either intact or broken, were incubated in the designated system with the indicated labeled compound for 1 hour at 37°C. After the incubation, 2 ml of 30 percent trichloroacetic acid (TCA) was added, and each assay tube was centrifuged at 2000*g*; the pellets were washed three times with 10 percent TCA and once with a mixture of ethanol and diethyl ether (2:1 by volume). The resulting pellet was dissolved in 1.0*N* NaOH, a portion was plated on a glass filter, and the radioactivity was determined by counting in a Beckman scintillation counter.

^{14}C-labeled compound	Intact mitochondria				Broken mitochondria system		
	Complete system *	0-Time control	Boiled control	RNase †	Complete ‡	$MnCl_2$ §	EDTA ||
Leucine ¶	1378	1	52	1552	461	200	34
GDP-Fucose #	270	4		350	126	74	7
GDP-Mannose #	436	17	31	308	344	387	42
UDP-Glucose #	695	12	56	684	720	610	14
UDP-Galactose #	288	6	12	337	294	377	13
UDP-Xylose #	2						

* Intact mitochondria prepared with sterile solutions were incubated in air in a metabolic shaker. The medium contained 10 m*M* $MnCl_2$, 5 m*M* sodium phosphate (*p*H 7.6), 50 m*M* tris(hydroxymethyl)amino methane (*p*H 7.6), 5 m*M* phosphoenol pyruvate, 20 μg of pyruvic kinase, 2m*M* adenosine triphosphate, 2 m*M* EDTA, 22.5 mg/ml of a complete amino acid mixture minus leucine, 7 to 15 mg of isolated mitochondrial protein, and 0.154*M* KCl to a final volume of 2.0 ml. † Ribonuclease was present at a final concentration of 0.5 mg per milliliter of incubation mixture. ‡ Complete system as above except that the mitochondria were broken with 24 strokes of a Ten Broeck homogenizer before assaying. § Each assay tube contained the isotopically labeled compound, 7 to 15 mg of broken mitochondrial protein, and 10 m*M* $MnCl_2$, to a final volume of 2.0 ml. || Each assay tube contained the isotopically labeled compound, 7 to 15 mg of broken mitochondrial protein, and EDTA, to a final concentration of 1 m*M*. ¶ Uniformly labeled L-leucine-^{14}C (0.5 μc) (250 c/mole). # Uniformly labeled monosaccharide (0.1 μc) was present as the nucleotide diphosphate monosaccharide; the specific activity of each nucleotide diphosphate monosaccharide was approximately 140 c/mole.

Table 2. Enzyme and radioactivity specific activity of isolated mitochondria. Mitochondria were isolated (*14*) and washed five times with 0.25*M* sucrose. Data are given as specific activity: units (μmole/hour) per milligram of mitochondrial protein. For the incorporation of leucine-^{14}C and monosaccharide-^{14}C, the assay was in each instance for the complete system with intact mitochondria as in Table 1, except the final volume was 3 ml instead of 2 ml. Data given as counts per minute per milligram of mitochondrial protein. Mitochondria numbered in the table refer to the successive washing with 0.25*M* sucrose.

Fraction	Enzyme activity				Radioactivity (^{14}C)			
	Esterase	UDPase	5′Nucleotidase	Monoamine oxidase	UDP-glucose	UDP-galactose	GDP-mannose	Leucine
Nuclear pellet	140	1.07	0.67	5.8				
Mitochondria 1	140	1.27	1.63	5.7	506	166	180	1000
Mitochondria 2	130	0.87	0.98	7.6	216	97	195	486
Mitochondria 3	41	0.09	0.08	7.2	263	76	104	207
Mitochondria 4	12	0*	0	11.9	553	93	250	265
Mitochondria 5	0	0	0	14.0	720	295	445	635
Mitochondria 6	0	0	0	16.8	832	319	361	593

* Zero indicates no activity was detectable using the method described when tested at highest concentration of mitochondrial protein possible.

fold increase in the concentration of ribonuclease had no effect on the incorporation of the monosaccharides. For each incorporated monosaccharide, the contents of two assay tubes were pooled and precipitated; the precipitate was then washed (Table 1); the homogenates were hydrolyzed in 1 ml of 1*N* HCl at 100°C for 3 hours, dried, and chromatographed in a mixture of butanol, pyridine, and 0.1*N* HCl (5:3:2, by volume) for 44 hours. Ninety-five percent of the radioactivity bound to the protein in the guanosine diphosphate (GDP)-fucose-^{14}C system was found as fucose; 70 percent of the radioactivity in the GDP-mannose-^{14}C system was recovered as mannose-^{14}C and 30 percent as glucose-^{14}C; 95 percent of the radioactivity in the uridine diphosphate (UDP)-glucose-^{14}C was recovered as glucose-^{14}C; and 95 percent of the radioactivity in the UDP-galactose-^{14}C was recovered as galactose-^{14}C.

Homogenization of the mitochondria (Table 1) decreased the incorporation of leucine-^{14}C to about one-third of the activity found in intact mitochondria. This result is consistent with the hypothesis that breaking of the mitochondria destroys structural elements of the mitochondria necessary for the incorporation of amino acids into protein; with only $MnCl_2$ present, the incorporation of leucine-^{14}C into protein was 15 percent of the amount incorporated by intact mitochondria. The incorporation of the monosaccharides into glycoprotein, except in the case of GDP-fucose-^{14}C, decreased by no more than 25 percent in the broken mitochondria or in the $MnCl_2$ system. In some instances the incorporation of monosaccharides into glycoprotein increased in either the complete system of broken mitochondria or the $MnCl_2$ system of broken mitochondria. These results indicate that the incorporation of the monosaccharides (except fucose) into glycoprotein was not dependent on the maintenance of intact structural elements of the mitochondria. $MnCl_2$ or another metal ion was necessary for the transfer of monosaccharide into glycoprotein; ethylenediaminetetraacetic acid (EDTA) (1 m*M*) inhibited the transfer (Table 1).

Mitochondria prepared as above were free of the following enzymes (Table 2): esterase (*15*), which is thought to be associated with microsomes (*16*); uridine diphosphatase (*17*), thought to be associated with smooth internal membranes (*18*); and 5′nucleotidase (*19*), thought to be associated with plasma membranes (*20, 21*). The washed mitochondria showed an increase in monoamine oxidase (*22*), an enzyme associated with mitochondria when expressed per milligram of protein (*23*). Furthermore, with each monosaccharide tested and in each instance of leucine transfer, the specific activity of the products increased with the mitochondrial purity from mitochondrial fraction number three to mitochondrial fraction number six, paralleling the increase in monoamine oxidase activity found in these fractions (Table 2). To confirm the macromolecular glycoprotein character of the radioactive product, the product was solubilized and purified.

Table 3. Solubilization and purification of radioactive product. Intact mitochondria were incubated with UDP-glucose-^{14}C in the complete system as given in Table 1 except that 1.0 μc of leucine-^{3}H was also added. Twenty-four samples were incubated and pooled and treated as given in the text. At each step the glycoprotein was precipitated with an equal volume of 50 percent TCA and washed with a mixture of ethanol and ether (2:1 by volume); the material assayed was TCA insoluble and not small peptides. Data given as counts per minute per milligram of Lowry protein. Two-milliliter fractions were collected from the G-75 column.

Fraction	Radioactivity (count min^{-1} mg^{-1} protein)	
	Leucine-^{3}H	UDP-glucose-^{14}C
Homogenate supernatant	366	570
Homogenate pellet	(130)	(190)
Dialyzate I	420	420
105,000*g* supernatant	482	650
105,000*g* pellet	(190)	(170)
Heat supernatant	640	840
Ethanol precipitate	730	1200
Dialyzate II	1400	1600

Sephadex G-75 fraction No.	O.D. (280 nm)	Leucine-^{3}H	UDP-glucose-^{14}C
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	0.021	0	0
7	0.423	486	540
8	3.500	1890	1820
9	3.900	2100	1190
10	3.200	942	1180
11	1.050	760	735
12	2.005	308	474
13	2.360	0	0
14	2.033	0	0
15	1.348	0	0
16	0.749	0	0
17	0.374	0	0
18	0.148	0	0
19	0.860	0	0
20	1.450	0	0
21	2.510	0	0
22	1.200	0	0
23	0.760	0	0
24	0.076	0	0
25	0.035	0	0
26	0.046	0	0
27	0.029	0	0
28	0.032	0	0
29	0.042	0	0
30	2.292	0	0
31	0.022	0	0
32	0.043	0	0
33	1.442	0	0
34	0.032	0	0

Since glucose-^{14}C from UDP-glucose-^{14}C was incorporated to the greatest extent, 24 assay tubes were incubated with the complete medium and intact mitochondria in the usual manner with UDP-glucose-^{14}C plus leucine-^{3}H (1.0 μc per assay tube). After incubation for 1 hour the mitochondria were centrifuged at 37,500*g* for 10 minutes and washed once with 0.25*M* sucrose. The following procedure was then performed at 4°C, unless otherwise indicated. The labeled mitochondria were homogenized in a Ten Broeck homogenizer (30 strokes) in 10 volumes of 0.4 percent sodium deoxycholate and the mixture extracted for 8 hours. The insoluble material was centrifuged at 2000*g* for 15 minutes. The supernatant material was decanted and saved while the pellet was extracted in 10 volumes of distilled H_2O for 8 hours. The two supernatants (homogenate supernatant in Table 3) were combined and exhaustively dialyzed against distilled H_2O for 24 hours with one change at 12 hours. The dialyzed material (dialyzate I in Table 3) was centrifuged at 105,000*g* for 30 minutes and the pellet was suspended in H_2O and centrifuged again at 105,000*g* for 30 minutes. The washed pellet is referred to above as "105,000*g* pellet" and the combined supernatants as "105,000*g* supernatant" in Table 3. The combined supernatants (80 ml) were evaporated to 5 ml at 80°C, and the material which precipitated was removed by centrifugation at 10,000*g* for 10 minutes and washed twice with water. To the combined supernatants (heat supernatants in Table 3), anhydrous sodium acetate was added to make a 1 percent (weight to volume) solution; then 5 volumes of absolute ethanol were added. The resultant precipitate was collected by centrifugation after standing at 4°C for 16 hours (ethanol precipitate in Table 3). The precipitate was dissolved in H_2O and exhaustively dialyzed against distilled H_2O for 16 hours; the dialyzate (dialyzate II in Table 3) was placed on a Sephadex G-75 (medium) column

(1.5 by 20 cm) and eluted in 2 ml fractions with H_2O at a flow rate of 0.5 ml per minute. The data (Table 3) show the glycoprotein nature of the radioactive product, and also indicate that the leucine and glucose are being incorporated into proteins which behave similarly in the solubilization procedure.

To determine the submitochondrial localization of the incorporated leucine-^{14}C and monosaccharide-^{14}C, inner and outer mitochondrial membranes were prepared by the Ficoll discontinuous gradient method (*24*). The majority of the radioactivity in each instance was found in the mitochondrial inner membranes. The counts per minute per milligram of protein in the inner membrane and the outer membrane were as follows: leucine-^{14}C, 1008 and 43; GDP-fucose-^{14}C, 317 and 0; GDP-mannose-^{14}C, 1180 and 289; UDP-glucose-^{14}C, 1900 and 159; and UDP galactose, 1720 and 0, respectively. The differential localization of leucine-^{14}C in inner membranes confirms previous observations (*6, 7*); the preferential incorporation of the monosaccharides-^{14}C into inner mitochondrial membranes indicates that the proteins to which the glycosyl residues are independently transferred by the isolated mitochondria are most likely the proteins which are independently synthesized by the isolated mitochondria. Although these results do not definitely rule out the possibility of smooth membrane contamination in the mitochondrial preparation, it seems unlikely that glycoprotein synthesized by smooth membrane fragments would be incorporated into mitochondrial inner membranes in this system in which cellular structural integrity is not preserved.

The results demonstrate that intact isolated mitochondria transfer monosaccharides from nucleotide diphosphate monosaccharide precursors into protein, thus creating glycoproteins. Most of the monosaccharide-^{14}C activity was found in the inner membranes, an indication either that the glycosylations occur in these membranes or that after glycosylation the glycoproteins are incorporated into these membranes. Thus, not only can the mitochondria synthesize a portion of their own proteins, but also the isolated mitochondria can synthesize their own glycoproteins independently of other cellular structures. These conclusions do not contradict those (*25, 26*) in which the smooth internal and plasma membranes of the cell were implicated as sites of cellular glycoprotein biosynthesis; rather they illustrate the ability of mitochondria to carry out some glycoprotein biosynthesis.

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Homing in the Ant Cataglyphis bicolor

Abstract. Cataglyphis bicolor, *an ant widely distributed in North Africa and the Near East, orient to the sun as well as to visual patterns of the environment. These two mechanisms can be separated. Foraging ants (hunters) orient to terrestrial cues as long as possible, and only after these have become ineffective do they switch over to the menotactical sun orientation. In the digging individuals, however, the visual knowledge of locality is significantly inferior to that of the hunters. Diggers vary considerably in size, but hunters belong to the largest size group. In addition, the largest and smallest individuals orient differently toward black and white areas and stripe patterns.*

Since the studies of Santschi (*1*), Cornetz (*2*), and Brun (*3*) there have been few investigations of optical orientation in ants (*4*). Their studies prove that at least some species of ants can use a time-compensated orientation to the sun's position or to the pattern of polarized light of the sky. However, an ant is unable to return to the nest menotactically after being displaced in any direction from its normal course. In such situations, visual landmarks must act as cues for optical orientation.

In our experiments on the optical orientation of ants during March and April 1968 in Israel, we studied *Cataglyphis bicolor* (var. *nigra*), a common ant of desert areas of northern Africa and southwest Asia. This species inhabits underground nests, which are indicated on the surface only by small holes surrounded by sand dikes and aggregated in little colonies. As a predatory and solitary hunter, the ant depends exclusively upon visual orientation. We demonstrated that after the ants are displaced, neither chemical nor vibrational stimuli are decisive as orientation parameters.

Displacements were first devised to put the already learned menotactical course in competition with that to known landmarks (*5*). Only hunters foraging from one definite direction (zero direction) were used. This zero direction (0° in all figures) coincides with that from which most hunters returned from their feeding places, at least 40 m away. One day before the test, all hunters used were individually color-marked. On their return from the foraging grounds (0°, west), they were intercepted 0.5 to 1.5 m from the nest entrance and captured in small plastic tubes; the ants were then shifted by 180° in the opposite direction (east) and released at various distances from the nest. With the use of a grid of fine thread (mesh width, 1 m) which extended over the whole experimental area, we recorded and timed the runs of the ants.

The percentage of positive runs (runs