Microsome-Associated DNA

Abstract. *Deoxyribonucleic acid has been isolated from the microsomes of mouse liver homogenates under conditions designed to prevent or greatly reduce mitochondrial and nuclear contamination. The DNA rapidly incorporates tritiated thymidine, and this, together with its reannealing characteristics after thermal denaturation, shows that it is not mitochondrial or typically nuclear DNA.*

In earlier studies on mitochondria (*1*), we observed a microsome-associated DNA which incorporated thymidine at a rapid rate. This suggested that its presence was not from nuclear contamination. We now have additional information and corroboration that this is a unique form of DNA associated with microsomes (*2*).

Microsomes and mitochondria were isolated initially with the A-IX zonal rotor (*3*). A mouse liver homogenate (20 percent in 0.25*M* sucrose) was prepared with minimum nuclear damage (*4*), and a 30-ml sample was introduced into the rotor between an 1100-ml gradient (13 to 40 percent sucrose) and 90 ml of overlay. The contents were centrifuged for 50 minutes at 3900 rev/min and then displaced out into 30-ml fractions. The initial sample zone was in tube 4, and the location of the microsomes ranged mainly from this tube through tube 9. Between tubes 9 and 12 there was a transition zone from microsomes to small mitochondria, and most of the mitochondria were found between fractions 12 and 28. Unbroken cells, nuclei, and membranes were predominantly beyond fraction 35 in the small amount of 55 percent sucrose cushion introduced between the gradient and the rotor wall.

Chemical analyses for DNA and protein (*5*) were performed on each fraction of the gradient, and ratios of DNA to protein (micrograms of DNA to milligrams of protein) were calculated. The RNA determinations (*6*) confirmed findings by electron microscopy that most of the microsomes were in fractions 5 through 9. The ratios of DNA to protein were lowest in tube 5, ranging from about 0.15 in this tube up to about 0.5 in tube 9. The ratio through most of the mitochondrial region was about 0.7 to 0.8, increasing slightly in the higher tube numbers where a small amount of nuclear DNA contamination was evidenced. These figures show that there was no major nuclear DNA contamination in the lower numbered tubes, a contention supported by the fact that nuclear satellite DNA was absent or was present only in trace amounts (*7*). The data also show a comparatively large amount of DNA associated with the microsomes.

Radioactive thymidine incorporation was determined on DNA isolated from the gradient fractions. Female Swiss mice (about 25 g) were each injected intraperitoneally with 350 μc of thymidine-methyl-^{3}H (6 c/mmole, specific activity) and killed 2 hours later. Liver homogenates were prepared and centrifuged with the A-IX rotor. Samples 4, 5, and 6 were pooled, and all remaining adjacent samples were pooled in sets of two for DNA extraction. The cellular constituents were centrifuged from 0.3*M* perchloric acid in the cold. The perchloric acid was immediately removed from the sedimented pellets with alcoholic potassium acetate (*5*); this was followed by lipid extraction. The extracted material was incubated overnight (16 hours) with predigested pronase, 1 mg/ml in 0.15*M* NaCl and 0.015*M* sodium citrate (SSC) with 1 percent sodium lauryl sulfate; the material was then digested for 1 hour with heat-treated pancreatic ribonuclease adjusted to 20 μg/ml. Alcohol was added to the solutions, and the DNA was centrifuged overnight in the Spinco-30 rotor. The pellets were redissolved in SSC, adjusted to a density of 1.7 g/ml with cesium chloride, and isopycnically banded twice in the Spinco-40 rotor (*8*). Portions were taken from the second cycle for chemical analysis, and radioactivity in the isolated bands was determined in a liquid scintillation counter with toluene–Triton X-100–Liquifluor (*9*); the counting efficiency was 9 percent. The radioactivity in the remaining portions of the gradients was also counted; the results showed that most of the radioactivity was associated with the DNA bands.

The DNA with the highest specific activity was obtained from the microsomal region. That from combined fractions 7 and 8 had the greatest activity [376 disintegrations per minute (dpm) per microgram]. The average activity in the mitochondria was 137 dpm/μg (range 110 to 171). The data indicate that the microsome-associated DNA is metabolically active and that it appears to be incorporating thymidine at a rate even greater than that of the mitochondrial DNA. Spurious incorporation from terminal labeling or a repair mechanism has not been excluded. However, we do not feel that such an artifact exists because there is no evidence for it in mitochondrial and nuclear DNA. Incorporation into mitochondrial DNA is within expected limits, and, although incorporation into nuclear DNA was not measured in this work, other studies have shown that very little would occur with the conditions used here.

Assuming that DNA contamination in microsomes, if present, would be mainly from broken nuclei and small mitochondria, we have conducted experiments designed to reduce or eliminate such constituents by isopycnically banding the microsomes before DNA isolation. Fractions 5 through 9 from the A-IX rotor were combined and in-

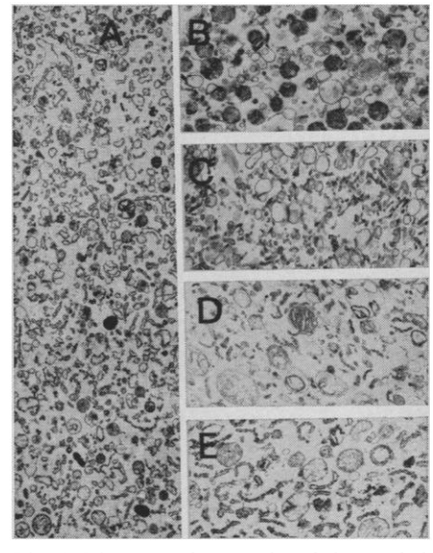

Fig. 1. Electron micrographs of the pooled subcellular constituents found in fractions 5 through 9 from the A-IX zonal rotor and the isopycnic bands obtained after centrifuging this material in the B-XIV rotor. (A) Pooled A-IX rotor fractions consisting almost entirely of microsomes, lysosomes, and microbodies (small mitochondria are rarely seen in these preparations) (× 5,000). (B) Uppermost or lightest band. With the mouse fraction we find the lysosomes in this position together with some membranous material (× 10,000). (C) Second band consisting of highly purified smooth endoplasmic reticulum (× 10,000). (D) The area of the gradient where mitochondria normally band. Although no band was seen at this location, a few small mitochondria were present, together with material from the bands on either side of this region (× 10,000). The material represented by (D) was discarded to reduce contamination from mitochondrial DNA. (E) Typical dense band consisting of rough endoplasmic reticulum and microbodies (× 10,000). This was a broad and diffuse band, probably a reflection of varying numbers of ribosomes associated with the membranes. Bands (B), (C), and (E) were combined before DNA extraction.

troduced into the B-XIV titanium zonal rotor (*10*) between a sucrose gradient (26 to 52 percent) and 100 ml of a sucrose overlay (10 percent) and centrifuged 2½ hours at 39,000 rev/min. Figure 1 shows electron micrographs of the starting material and the banded fractions recovered from the rotor. Three distinct bands were present. A transitional area located between the second and third band (numbered from the top of the gradient) contained any small mitochondria which were present. This region (Fig. 1D) was discarded before combining the three bands remaining for DNA extraction.

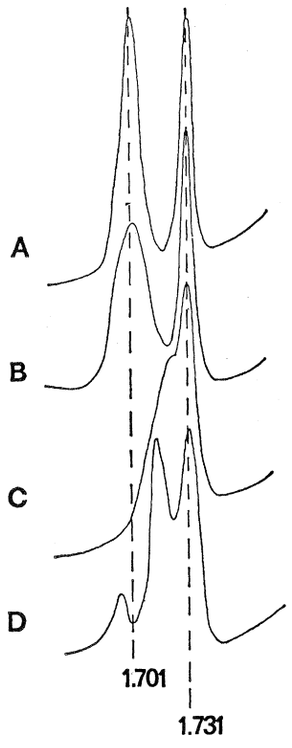

Fig. 2. Densitometer traces of DNA banded in cesium chloride in the model E ultracentrifuge equipped with ultraviolet optics. The materials were centrifuged 20 to 24 hours at 44,000 rev/min at 25°C. The band at 1.731 is a *Micrococcus lysodeikticus* marker. (A) DNA extracted from the pooled material described in Fig. 1. Note the absence of a satellite DNA peak. The band is destroyed by deoxyribonuclease but is not affected by ribonuclease. (B) The same material as (A) after brief sonication. Material treated in this manner was used for the subsequent heat denaturation and reannealing experiments. (C) The sonicated DNA after thermal denaturation at 100°C for 10 minutes in one-tenth concentrated SSC and quick cooling in ice. (D) DNA treated as in (C), then adjusted to standard SSC and incubated at 60°C for 24 hours. The small peak has renatured much as nuclear satellite would, although (A) indicates that satellite may be absent. Considerable reannealing has occurred in the major component, as evidenced both by band movement and sharpening of the component.

Figure 2 shows the cesium chloride banding characteristics of the DNA from the combined fractions, and the results of thermal denaturation and reannealing experiments with the material. The native density of the DNA is indistinguishable from both mitochondrial DNA and the mouse nuclear DNA major band. However, satellite DNA (*7*) comprising about 10 percent of the mouse nuclear DNA apparently is missing, because DNA from controls similarly tested show distinct sharp satellite bands at a density of 1.691. The absence of the satellite in addition to the metabolic activity of the DNA indicates that its presence is not from nuclear contamination. Material similar to nuclear satellite DNA was found in the reannealing experiment (Fig. 2D). A small component reannealed to a density of 1.697. However, since this large an amount of nuclear satellite DNA is clearly absent, it is probable that a new mouse satellite DNA has been demonstrated by the experiment.

In the reannealing experiment shown in Fig. 2, the microsome-associated DNA is not mitochondrial, because the latter anneals more readily and more completely. An intermediate buoyant density was found after 6 hours of incubation, an indication that the annealing process was still progressing at this time.

We have demonstrated a unique form of DNA associated with microsomes. The material is not mitochondrial. If it should come from nuclear contamination, it would represent a form of nuclear DNA heretofore unknown. The DNA appears metabolically active, and, based upon its reannealing characteristics, it is quite complex and contains potentially a considerable amount of genetic information.

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References and Notes

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4. The livers were put through a tissue press, and homogenized (20 percent on a weight-to-volume basis with 0.25*M* sucrose) in a Dounce homogenizer with seven slow, downward passes with a loose-fitting pestle. Most of the cells were broken, but a considerable number of intact cells were observed by phase-contrast microscopy. Although some tissue was lost because of incomplete homogenization, the possibility of nuclear damage was minimized, and so was the possibility of nuclear DNA contamination in nonnuclear fractions.
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Blastocladia and Aqualinderella: Fermentative Water Molds with High Carbon Dioxide Optima

Abstract. *The uniflagellate aquatic phycomycete* Blastocladia ramosa *appears to be a facultative anaerobe.* Blastocladia pringsheimii *requires traces of oxygen. Growth of both species is no greater or only slightly greater at normal atmospheric oxygen pressure than under 0.2 percent oxygen pressure, but their growth is enhanced by the addition of 5 or 20 percent carbon dioxide. The cells of both species lack typical cristate mitochondria and contain only traces of cytochrome.* Blastocladia *resembles the biflagellate* Aqualinderella fermentans *in adaptation to an environment poor in oxygen and rich in carbon dioxide.*

The existence of a polyphyletic group of fermentative water molds adapted to anaerobic environments rich in CO_2 has been suggested (*1*). We now confirm the occurrence of such an ecological pattern among uniflagellate phycomycetes and provide further data about their biflagellate counterparts.

Fungi are generally regarded as obligate aerobes (*2*). In facultative anaerobes such as *Saccharomyces cerevisiae* (*3*) and *Mucor rouxii* (*4*) the removal of oxygen causes a loss of mito-