that remains more or less constant in thickness.

This implies that morphogenesis proceeds by intercalation of materials into this ring, rather than by a "stretching" process. Previously, this ring was thought to be siliceous in umbellate specimens (*3*). No intermediate between the tripartite stage and the "mature" forms with six to eight parts has been observed; however, these preumbellate forms are rarely seen in cultures in which umbellate structures are relatively abundant. (The tripartite structure has never been found with the more mature stages.) After prolonged cultivation, the count of intact umbellate forms began to decline, eventually falling to zero, and the mature form disintegrated after several weeks in culture. Specimens showing ring and spoke structures in the process of disintegration have been seen (Fig. 5). Because of the absence of a logarithmic phase in growth curves and the failure, during many hours of visual observation, to note any signs of a fission process, we conclude that the ontogeny of this organism is complex and involves a distinct reproductive phase. This phase may have gone unrecognized because of the organism's small size and a structure that renders it difficult to distinguish from the larger motile bacteria. Even the earliest presumptive developmental stage (Fig. 4) could not in itself be identified with the later tripartite form were it not for the suggestive intermediate structures. A possible ontogeny for these *Kakabekia*-like forms is:

Small (about 1 μ) heavy-walled ellipsoid form (cyst, zygospore, zoospore?)
↓
Enlargement 5 to 10 times and radial segmentation (mitosis or meiosis?)
↓
Stalk (stipe) "primordium"
↓
Mature umbellate form with stalk and bulb
⟶ gametogenesis or sporogenesis
↓
Senescence and degeneration

The material within the spokes or septa of the umbrella gave a positive reaction with both Feulgen and acetoorcein stains, and the stalk was sometimes "cellular" in appearance. No organized nuclear structure has been observed, however. Conceivably, genetic material from the umbrella migrates into the stalk, aggregates in the basal bulb, and is released as a reproductive stage.

This ontogenetic picture differs from that proposed by Barghoorn and Tyler (*2*), which was based on arrangement of their fossil specimens. It is, essentially, the inverse of their sequence which begins with the basal bulb as the direct progenitor of the umbellate stage. Both ontogenetic sequences are subject to the same limitations on actual observation of developmental processes in pure culture or in the field. It can be hoped that the "living fossil" will eventually resolve this problem.

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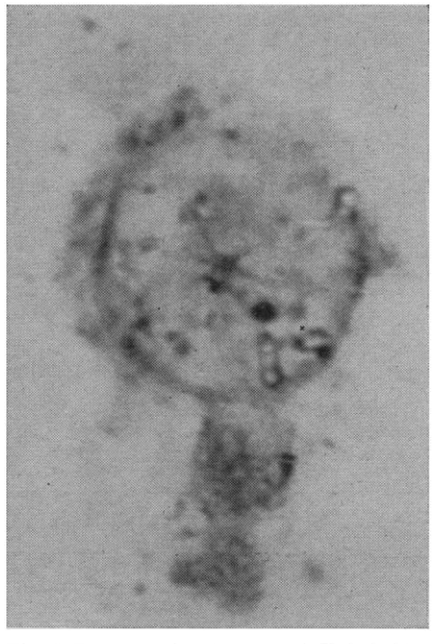

Fig. 5. Degenerating (senescent) *Kakabekia*-like form from a 6-week-old culture. (× 1250)

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Mitochondrial-Satellite and Circular DNA Filaments in Yeast

Abstract. *Mitochondrial DNA of* Saccharomyces cerevisiae *contains a satellite DNA (density, 1.682) that appears to exist as open-ended filaments at least 5 microns long. DNA from intact cells contains circular filaments whose lengths vary from 0.5 to 7 microns, with a great majority at 1.95 microns. The circular DNA has a density similar to that of the major nuclear peak (1.697). When heat-denatured mitochondrial-satellite DNA is renatured, it cross-links to form a molecule that is larger than the native molecule. The formation of cross-links results in hypersharpening of the density profiles in cesium chloride and also leads to failure to pass Millipore filter paper.*

Genetic and cytological evidence (*1*) of the presence of DNA that is not physically integrated into the nuclear genome has recently been confirmed; the evidence has been extended to the extraction and purification of organelle-specific DNA from chloroplasts (*2*) and mitochondria (*3*, *4*). This type of DNA is of relatively small molecular size. The DNA isolated from mitochondria of several higher organisms is circular (*5*, *6*). DNA obtained from yeast mitochondria is lighter in density than nuclear DNA. Our new data suggest that mitochondrial-satellite DNA is not circular but in a linear form about 5 μ long. Circles of varying sizes, however, have been detected in DNA either extracted from intact yeast cells or obtained from mitochondrial preparations, but they are present in the heavier DNA, of nuclear density, that is present in both preparations. Mitochondrial-satellite DNA that had been denatured and renatured has also been examined by electron microscopy; it proved to be extensively cross-linked and to contain denatured portions of the molecule; consequently it manifests hypersharpening of its profile in CsCl-gradient centrifugation and does not pass Millipore filters.

A strain of commercial (Red Star brand) *Saccharomyces cerevisiae* was aerated to stationary phase in a medium consisting of 2-percent Difco

Bacto-yeast extract, 1-percent Difco Bacto-peptone, and 1-percent glucose. Protoplasts were prepared with snail digestive juice (*7*) prior to disruption of cells for mitochondrial isolation or DNA extraction. The protoplasts were suspended in a mixture of 0.25*M* sucrose and 0.0005*M* ethylenediaminetetraacetate (*p*H 7.0) and homogenized in a motor-driven Potter-Elvehjem tissue grinder. Mitochondria were prepared by differential centrifugation (*7*) and purified by equilibrium centrifugation in a two-step sucrose gradient. The mitochondria banded at the interface of 0.6*M* and 1.5*M* sucrose, while nuclei sedimented at the bottom of the tube. DNA was extracted with phenol, digested with ribonuclease, and either dialyzed against saline sodium citrate solution (0.15*M* NaCl, 0.015*M* sodium citrate) or precipitated with ethanol to remove nucleotides (*4*). The DNA was further purified and fractionated by isopycnic density-gradient centrifugation in CsCl. Similar procedures were used to extract DNA from whole cells. The density of purified DNA was determined (*8*). Phage SPO-1 DNA (ρ, 1.740) was used as a reference marker. DNA was prepared for electron microscopy by spreading solutions containing DNA (5 to 10 μg/ml) in 1*M* ammonium acetate and 0.01-percent cytochrome *c* on an air-water interface, picking up the film on carbon-coated copper grids, and shadowing it with uranium oxide (*9*). The experimental procedures have been detailed (*10*).

DNA isolated from purified yeast mitochondria exists as a distinct satellite that has a density of 1.682—0.015 lighter than nuclear DNA (Fig. 1A); similar results have been reported (*11, 12*). The mitochondrial-satellite DNA is double-stranded, as indicated by increase in density by 0.015 after heating to 100°C for 10 minutes (Fig. 1B); it also renatures rapidly (Fig. 1C; *12*). From 50 to about 90 percent of DNA extracted from our mitochondrial preparations was of satellite density; the remainder was presumed to originate, at least in part, from contamination with nuclear DNA. Pure satellite DNA was obtained by subfractionation of the DNA, previously extracted from mitochondria, by preparative centrifugation in CsCl. The density of material in the denser or leading edge of the profile was the same as that of nuclear DNA, while the lighter side of the preparative peak contained DNA of satellite density.

Mitochondrial DNA that had not been subfractionated in CsCl, to remove contaminating nuclear DNA, contained a number of circular DNA filaments when viewed with the electron microscope. After subfractionation, circles were present only in fractions that contained DNA of nuclear density. Electron-microscopic observation of highly purified satellite preparations consistently showed the presence of filaments varying in length from 0.1 to 5.5 μ (Fig. 2A). Measurements of 175 filaments revealed no clear-cut grouping with respect to length. The presence of large numbers of filaments as long as 5.5 μ may be interpreted as resulting from breakage of molecules that had been at least 5 to 6 μ in the native state. The distribution of filament lengths was similar in preparations of mouse mitochondrial DNA (*6*), in which the distribution was attributed to breakage of 5-μ circles.

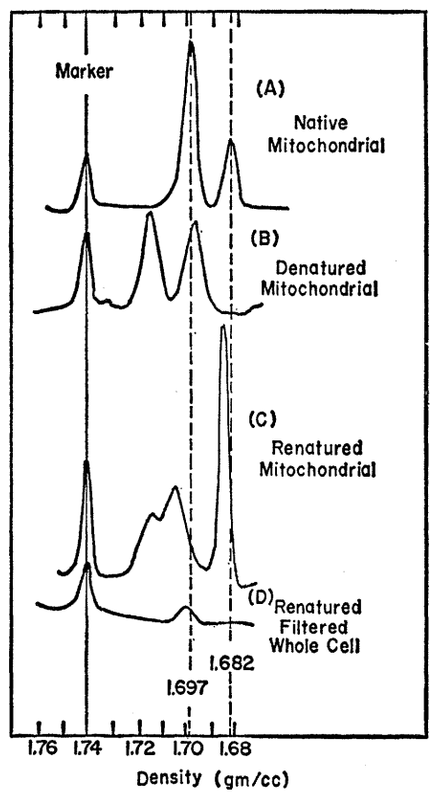

Fig. 1. Microdensitometric tracings of DNA photographed after 20-hour centrifugation at 44,770 rev/min on the analytical ultracentrifuge. (A) Native mitochondrial DNA (ρ, 1.682) with significant nuclear (ρ, 1.697) contamination; (B) similar DNA with less contamination after heating for 10 minutes at 100°C; (C) same as (B) after renaturation for 6 hours in 0.3*M* NaCl, 0.03*M* sodium citrate, at 60°C; (D) whole-cell DNA after heat denaturation, renaturation as in (C), and filtration through nitrocellulose (S&S type B-6) filters.

The filaments observed in yeast probably did not originate from breakage of circles during isolation and extraction, since intact circular molecules were observed in fractions of nuclear density (ρ 1.697). The presence of only a very few molecules longer than 5 μ suggests that 5 to 6 μ may be the native length, which corresponds to a molecular weight of 1×10^7 daltons and agrees with the size reported from sedimentation data (*11*); it resembles the size of DNA filaments extracted from *Neurospora* mitochondrial DNA (*3*) and of the circular DNA filaments present in mitochondria of mammals and chicks (*5, 6, 10*). The possible genetic content of a DNA filament having a molecular weight of 10^7 has been discussed (*6, 10*).

Circular filaments were present in DNA extracted from whole cells as well as in mitochondrial preparations that had not been subfractionated on CsCl to remove material of nuclear density; Fig. 2C shows the appearance of a number of these filaments. The proportion of total DNA in the form of circles was very small—probably less than 1 percent. We could not determine accurately the proportion of circular DNA molecules because of the relatively small number of circles.

Measurements of about 500 circular filaments showed variation in length from about 0.3 to 7 μ, with a highly significant majority in a class having an average length of 1.95 μ (Fig. 3). Other lengths occurring in sufficiently large numbers to constitute possible classes were 0.6, 1.0, 1.95, 2.8, 3.2, 3.7, and 5.0 to 5.5 μ.

In an attempt to determine the cellular location of the circles, distributions of circle lengths from mitochondrial preparations were compared to those from whole-cell preparations. The 1.95-μ class of circular filaments was present in significantly higher proportions in DNA preparations from intact cells than in mitochondrial extracts (Fig. 3). This observation suggests that circles longer than 1.95 μ may be relatively more numerous in mitochondria or membranous particulates than in the nucleus.

If small differences in length between classes are significant, it is possible that some specific classes (for ex-

ample, 2.8 μ) may be concentrated in mitochondrial extracts. We should point out that mitochondria prepared for these extractions were significantly contaminated with nonmitochondrial membranes; more-conclusive statements on localization of classes of circles must therefore await improvement of procedures for fractionating yeast-cell particulates.

In order to confirm that the circles observed were not artifacts of the method of preparation, DNA was isolated from yeast protoplasts by a procedure that did not use phenol extraction and was designed to minimize shear. Whole yeast protoplasts were lysed in 0.5-percent sodium dodecyl sulfate, adjusted to a density of 1.710 with CsCl, and centrifuged to equilibrium in a preparative ultracentrifuge (*13*). The total-DNA peak was collected in ten-drop portions. Each fraction was then examined both by electron microscopy and by analytical isopycnic ultracentrifugation. All circle sizes seen in phenol extracts were also present in these preparations. In fractions of higher density the 1.95-μ circle size was more highly concentrated than other circle sizes. There was no enrichment of any of the circle sizes in the fractions of lower density, which showed significant enrichment of mitochondrial-satellite DNA when examined by analytical isopycnic ultracentrifugation.

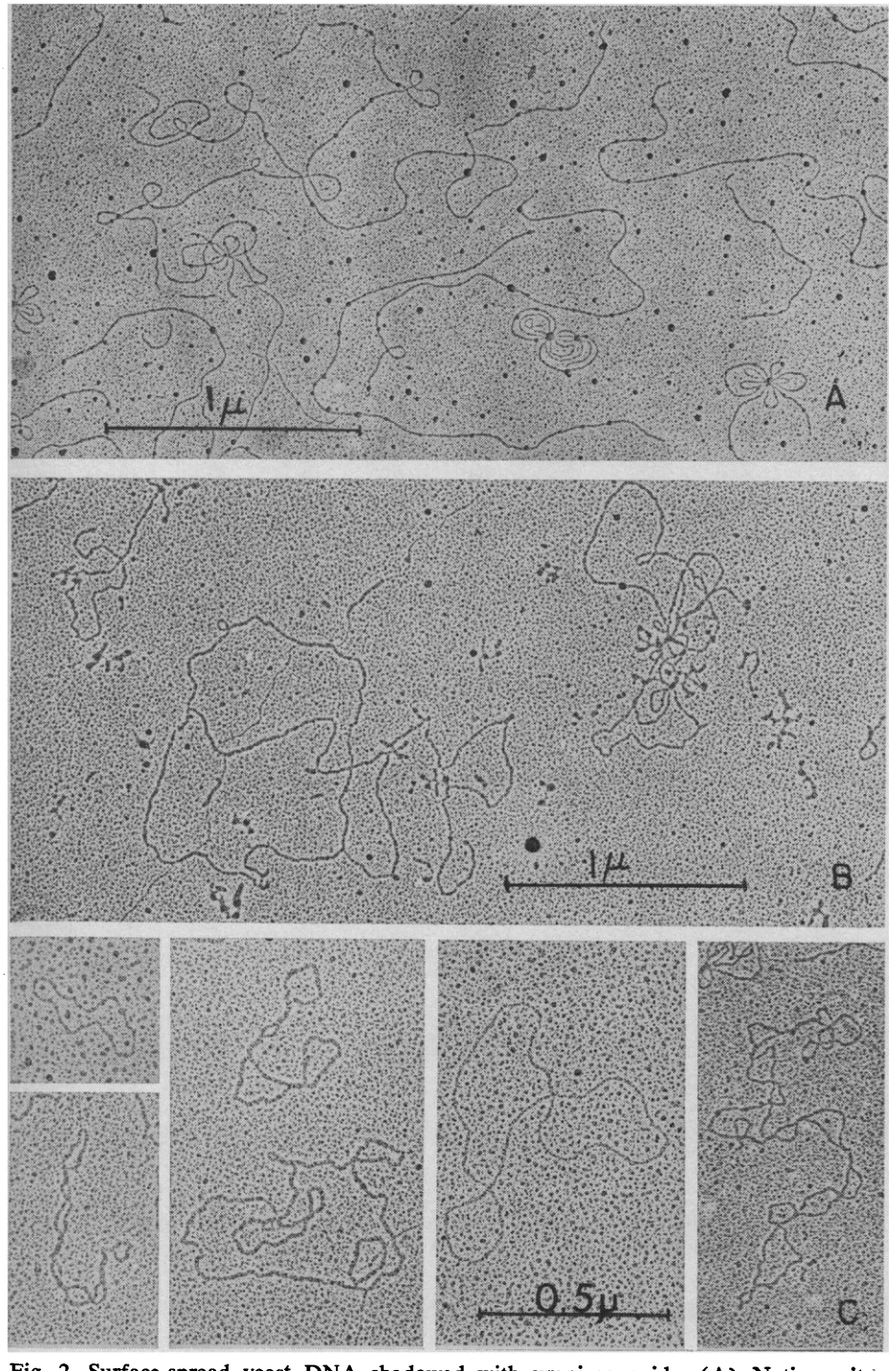

Fig. 2. Surface-spread yeast DNA shadowed with uranium oxide. (A) Native mitochondrial-satellite DNA purified by subfractionation with CsCl; (B) mitochondrial-satellite DNA after denaturation and renaturation; (C) various examples of circular DNA filaments from whole-yeast-cell DNA.

Although mitochondrial-satellite DNA appears to be noncircular, it is renatured more rapidly than nuclear DNA; it then forms sharper bands than does native satellite DNA in CsCl (Fig. 1C). A possible explanation of band sharpening is a decrease in the rate of diffusion, resulting from increased molecular size. When renatured satellite DNA was examined by electron microscopy, extensive branching, forking, and cross-linking were seen (Fig. 2B); cross-linking of several molecules increases molecular size. No evidence of circle formation upon renaturation was obtained.

Renatured DNA also fails to return completely to the original density of native DNA, remaining 0.002 to 0.003 denser (Fig. 1C). The presence in the electron micrographs of double-stranded molecules that have regions of "puddled" DNA suggests that the slightly higher density of renatured DNA is due to these denatured portions. ("Puddled" describes the collapsed or globular configuration often taken by single-stranded DNA spread on carbon grids.)

The presence of unrenatured portions is also indicated by the inability of renatured satellite DNA from yeast mitochondria to pass nitrocellulose filter paper (*10*); renatured DNA having the density profile shown in Fig. 1C was completely retained by such paper. This fact indicates that relatively small regions of denatured DNA effectively prevent filtration, although most of the molecule is double-stranded.

When milligram quantities of DNA extracted from whole yeast were heat-denatured, renatured, and passed through a nitrocellulose filter at a concentration of 100 μg/ml, a very small amount of material absorbing at 260 mμ could be detected when the filtrate was concentrated by pervaporation. The

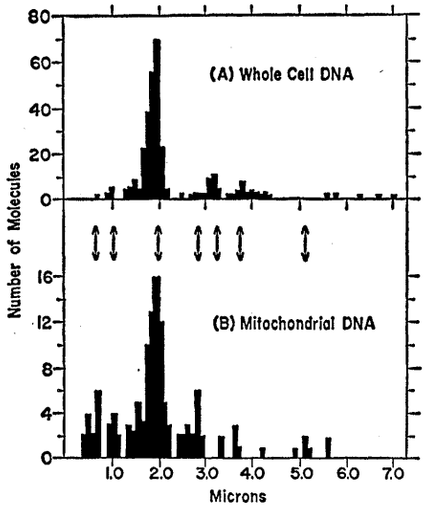

Fig. 3. Histograms of lengths of yeast circular DNA. Circles in DNA extracted from whole yeast cells (A) or from mitochondria prior to subfractionation in CsCl (B). Double-ended arrows indicate lengths of high-frequency classes.

material had a density of 1.701—slightly heavier than nuclear DNA; it may represent fragments of DNA that, while short enough to pass a nitrocellulose filter, still have sufficient denatured regions to make them denser. Another possibility is that this is 100-percent renatured DNA derived from the gamma or heavier satellite DNA present in yeast (*12, 13*). Electron microscopy showed that this filtrate contained both circles and short renatured rods; the circles were expected from the tendency of circular DNA to be renatured readily, but the composition of the short rods is unknown.

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Pyrimidine Nucleotide Synthesis: Regulatory Control during Transformation of Lymphocytes in vitro

Abstract. *The incorporation of uridine into RNA in lymphocytes undergoing transformation induced by phytohemagglutinin parallels its incorporation into the intracellular pool of acid-soluble nucleotides and coincides with a 20-fold increase in the specific activity of uridine kinase. This increase is dependent upon synthesis of both RNA and protein and is subject to both repression and end-product inhibition by cytidine.*

Lymphocytes from human blood undergo a striking morphologic transformation upon culture in the presence of phytohemagglutinin (PHA), a protein extracted from the kidney bean, *Phaseolus vulgaris* (*1*). The earliest biochemical changes preceding this transformation include an exponential increase in incorporation of uridine into RNA (*2*) and an increase in both the acetylation of histones (*3*) and the phosphorylation of histones and lipoproteins (*4*). Somewhat later, cells in culture produce gamma globulin (*5*), a variety of cellular enzymes active in glycolysis and oxidative phosphorylation (*6*), and a substance similar to interferon (*7*). The suggestion has been made that the process of transformation induced by PHA can be regarded as a nonspecific process of extensive gene activation (*4*).

I find that uridine kinase activity is induced in cells stimulated by PHA and that this process is subject to the types of regulatory control mechanisms of repression and end-product inhibition previously described for bacterial and mammalian cells (*8*).

Lymphocytes from human blood were purified by (i) a preliminary sedimentation by gravity after the addition of plasmagel (an amount equal to one-fourth the volume of blood) (*9*) to remove the bulk amount of erythrocytes; (ii) adsorption of the polymorphonuclear and other phagocytic cells onto a column of nylon fibers (*10*); and (iii) repeated sedimentation, by gravity, of samples concentrated by centrifugation to facilitate rouleaux formation of the erythrocytes. The final preparations, as judged from Wright staining, had only two polymorphonuclear and 8 to 20 red blood cells per 100 lymphocytes. The cells were cultured in Eagle's minimal essential medium (MEM) for suspension culture (supplemented with 15 percent fetal calf serum, glutamine, penicillin, and streptomycin) in a carbon dioxide

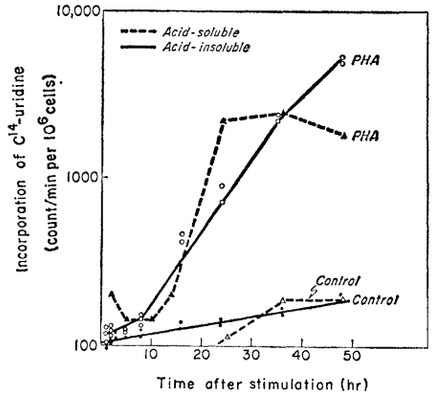

Fig. 1. Kinetics of ^{14}C-uridine incorporation into the intracellular pool of acid-soluble nucleotides and into RNA. Lymphocytes (5×10^6) in 2 ml of supplemented Eagle's MEM were incubated with 3.6 mμmole of ^{14}C-uridine (27.6 mc/mmole) for 2 hours at various times after the addition of PHA. Control cultures were labeled at the same time. The radioactivity in the acid-precipitated fraction retained on Millipore filters was determined in a liquid scintillation counter. The counts were normalized to 10^6 cells. The filtrate was collected quantitatively, and the radioactivity was determined from samples. Incorporation into RNA is indicated by solid lines; incorporation into the acid-soluble pool, by the dashed line.