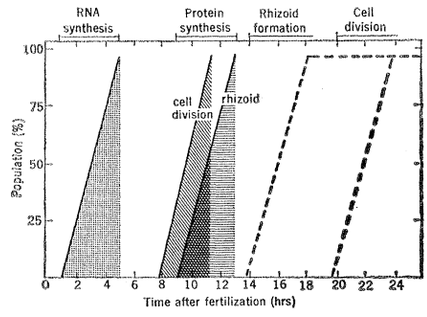

Fig. 2. Illustration of the timing of RNA and protein synthesis required for rhizoid initiation and cell division. Each shaded triangle represents the period during which a given percent of the population has synthesized an RNA or protein fraction essential for rhizoid initiation or cell division. Dashed lines indicate the normal time course of the zygote population passing through these two events.

tion of the cell's capacity to form rhizoids by cycloheximide is a result of a relatively specific block in protein synthesis (Fig. 2).

Experimentation with a number of diverse organisms has demonstrated that inhibitors exhibiting their effect at the level of transcription of RNA from DNA (actinomycin) must be given earlier than inhibitors acting at the translational level of protein synthesis (cycloheximide) to prevent a given growth or differentiative event (or both) (*7*, *19*). Such experiments provide evidence for the existence of control of protein synthesis at translation. Rhizoid formation in *Fucus*, like cap regeneration in *Acetabularia* (*10*), seems to be an example of a unicellular system demonstrating control of differentiation at the translation level of protein synthesis.

On the basis of similar experiments during animal embryonic development, Tyler (*7*) has proposed that the production of "masked forms" of RNA (*6*) occurs roughly at the stage when the eventual fate of certain multicellular regions becomes "determined." Tissue differentiation, then, would be equivalent to the eventual translation of this masked form of RNA into tissue-specific proteins.

In *Fucus* zygotes the cytoplasmic area destined to be the site of rhizoid formation is irreversibly fixed only 1 to 3 hours before rhizoid emergence (*2*). This corresponds more closely to the time when proteins, not RNA, required for rhizoid formation are synthesized. Therefore, in *Fucus*, it seems that the production of an essential RNA fraction for a given intracellular differentiative event does not correspond to "fixing" a cytoplasmic area to its future developmental fate. It is the synthesis of proteins, the timing of which seems to be controlled at the translation level, which irreversibly determines a specific region of the cytoplasm to initiate events quickly leading to a macroscopic polar cell. It is not known whether essential proteins are synthesized at the site of subsequent rhizoid initiation, or synthesized at random and transported to this site.

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DNA Biosynthesis by Isolated Mitochondria: A Replicative Rather Than a Repair Process

Abstract. *The previously observed incorporation of deoxynucleoside triphosphate precursors into DNA by isolated rat liver mitochondria could reflect either replication of DNA or a repair process. Density labeling experiments in cesium chloride demonstrate that DNA synthesized in the presence of 5-bromodeoxyuridine triphosphate instead of thymidine triphosphate shows an appreciable increase in density. In some of the molecules undergoing synthesis, the amount of the density increase indicated a replacement of thymine by bromouracil to the extent of 33 percent. This extensive replacement, which would compute to twice this amount if only a single strand of the duplex is labeled, provides evidence for the synthesis of fairly long pieces of DNA. Such synthesis is characteristic of replication rather than repair, and the results thus suggest that mitochondria are able to replicate their own DNA.*

Recent studies lend support to the view that mitochondria possess a remarkable degree of autonomy; they appear to contain some of the information and apparatus for macromolecular biosynthesis and possibly for their own biogenesis. They contain DNA (*1*), aminoacyl tRNA synthetases and tRNA (*2*), and ribosomes (*3*), all of which appear to be distinct from their nuclear or cytoplasmic counterparts; they have also been demonstrated to carry out the biosynthesis of protein (*1*). Although mitochondria are under the control of nuclear genes (*4*), they are under the control of cytoplasmic genetic factors as well (*5*). Recent evidence indicates that these genes are located in the mitochondrion (*6*), suggesting that they are identical with mitochondrial DNA. The

biogenesis of new mitochondria takes place by fission (*7*), and mitochondrial DNA replicates semiconservatively (*8*). However, these in vivo results on DNA replication (*8*) provide no evidence that mitochondrial DNA is replicated by or in the mitochondria.

Recently, direct evidence has been obtained for DNA synthesis by mitochondria. The evidence comes from the demonstration by Parsons and Simpson (*9*) that isolated rat liver mitochondria can effect the incorporation of labeled deoxynucleoside triphosphates into DNA, characterized both physically and chemically as mitochondrial DNA (*9*). More recently, Wintersberger obtained similar results with yeast mitochondria (*10*). In the rat liver experiments, the incorporation process required all four deoxynucleoside triphosphates and Mg^{+2}, and occurred in the absence of bacterial or nuclear contamination; the incorporated deoxynucleotide was found in the interior of the DNA molecule. The extent of incorporation was equivalent to the synthesis of about 1 percent of the amount of mitochondrial DNA present. Cesium chloride density gradient centrifugation of the labeled native, denatured, and renatured product established the identity of the DNA as mitochondrial. Fractionation of the product by CsCl centrifugation, with use of the ethidium bromide technique (*11*) shows that the DNA of the two fractions obtained, that is, superhelical DNA and open circular plus linear DNA, are labeled. Nearest neighbor frequency analysis provides no evidence for spurious or unusual base frequencies in the labeled DNA (*12*). Moreover, a DNA polymerase has been isolated from mitochondria which is distinct, differing in enzymatic and chromatographic properties from that located in the nucleus (*13*).

An assessment of the significance of the deoxynucleoside triphosphate incorporation in terms of mitochondrial autonomy depends to a great extent on whether this reaction is a reflection of a true replicative process, that is, the synthesis of new DNA strands, or merely mirrors a DNA repair mechanism (*14*, *15*). Thus, at one extreme, the 1 percent synthesis we observe could reflect the complete replication of new strands of DNA in an amount equivalent to 1 percent of the total number of DNA strands present; at the other extreme, it could reflect the repair of all DNA molecules present, to the extent of 1 percent of the length of each molecule. The limited degree of DNA synthesis during the incubation period does not permit a distinction to be made between these two processes, and the technique of density labeling was employed to clarify this question.

If, during the incubation, the DNA is permitted to incorporate 5-bromouracil (BrU) in place of thymine, the replication of wholly new strands, or the continuation of the synthesis of large portions of pre-initiated strands, should result in a substantial increase in buoyant density of the newly synthesized DNA molecules. On the other hand, if the synthesis we observe reflects the repair of 1 percent of the length of each DNA molecule on the average, the density increment resulting from the replacement of 1 percent of the thymine residues by BrU would be virtually undetectable on CsCl centrifugation. That rat liver mitochondrial DNA is capable of incorporating BrU is shown by studies on intact animals (*16*).

Mitochondria from rat liver were isolated and washed as described previously (*9*) and were incubated in the presence of 5-bromodeoxyuridine triphosphate (BrdUTP) (*17*), or thymidine triphosphate (TTP) as a control, tritium-labeled deoxyadenosine triphosphate (dATP-H^3), tritium-labeled deoxycytidine triphosphate (dCTP-H^3), and deoxyguanosine triphosphate (dGTP). (It was necessary to use the triphosphate of 5-bromouridine in the reaction mixture inasmuch as BrU itself is not incorporated into DNA under our reac-

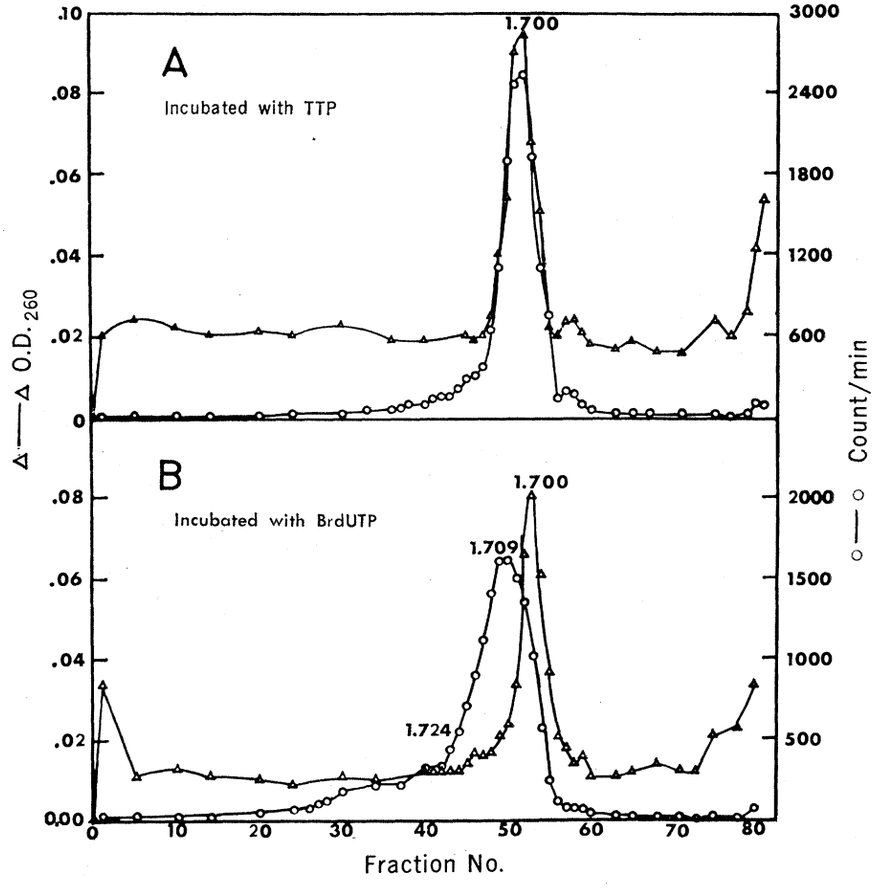

Fig. 1. Cesium chloride centrifugation of mitochondrial DNA labeled with BrU in vitro. The reaction mixture for the control sample (A) contained 4.0 m*M* KCl, 5.0 m*M* KH_2PO_4, 7.5 m*M* pyruvate, 7.5 m*M* succinate, and 0.45 m*M* malate. After adjustment of the solution to *p*H 7.4 with KOH, the following components were added: 2.0 m*M* $MgCl_2$, 15 μM dGTP, 0.5 μM dATP-8-H^3 (5.66 c/mmole), 0.12 μM dCTP-H^3 (26.0 c/mmole), 80 mg of mitochondrial protein, and 12 μM TTP. The final volume was 8.0 ml, and the reaction was carried out at 37°C for 90 minutes. In the experimental sample (B) 12 μM BrdUTP was substituted for the TTP. DNA was isolated by phenol extraction (*18*) and the CsCl centrifugation was performed in the Spinco SW65 rotor at 37,500 rev/min for 67 hours at 20°C. Samples were processed as previously described (*9*). Densities were determined with the Bausch and Lomb refractometer, and the gradient was calibrated from the known density of rat liver mitochondrial DNA (*19*). The counting efficiency was 5 percent.

tion conditions. The labeling of DNA which we have observed when either 5-bromouridine or thymidine was used is spurious; it can be washed out by rigorous purification procedures.) Following the incubation, the mitochondria were reisolated by centrifugation in the Sorvall SS-34 rotor at 8500 rev/min and the DNA was extracted by the phenol-sodium dodecyl sulfate procedure of Saito and Miura (*18*). The DNA preparation was then subjected to CsCl centrifugation. Control samples were incubated with TTP replacing BrdUTP, and were centrifuged concurrently. The results are shown in Fig. 1. The triangles are optical density plots representing bulk mitochondrial DNA. The circles, denoting the incorporated radioactivity, represent newly synthesized DNA. It can be seen that in the TTP control (Fig. 1A) the radioactivity and optical density peaks coincide exactly. On the other hand, the radioactivity peak in the BrU sample is clearly displaced from the O.D. peak by an amount equivalent to 0.009 g/cm^3; a marked increase in density of the newly synthesized DNA has occurred. Based on the data of Erikson and Szybalski (*19*), a 100 percent replacement of thymine by BrU in a DNA containing 30 percent thymine (which is the thymine content of rat liver mitochondrial DNA) corresponds to an increase in density of 0.062 g/cm^3. Assuming a linear relationship between percent of replacement and buoyant density, the density shift of 0.009 g/cm^3 corresponds to a replacement of 15 percent of the thymine residues in each double-stranded molecule undergoing deoxynucleotide incorporation.

The DNA repair processes that have been studied, both in bacteria and in mammalian cells, are thought to be limited in extent; they involve the relatively infrequent insertion of small numbers of nucleotides into damaged sections of the DNA molecule (*15*). The extent of such repair does not exceed about 1 percent of the length of the DNA molecule (*15*). Thus, the replacement of 15 percent of the thymine residues by BrU strongly suggests that new strands of DNA are being synthesized, pointing to the occurrence of a replication process.

If DNA replication is indeed occurring during incubation, it would be expected that the value of 15 percent for thymine replacement is an average value with some molecules having undergone synthesis to greater, and some to lesser, extents. The increased width of

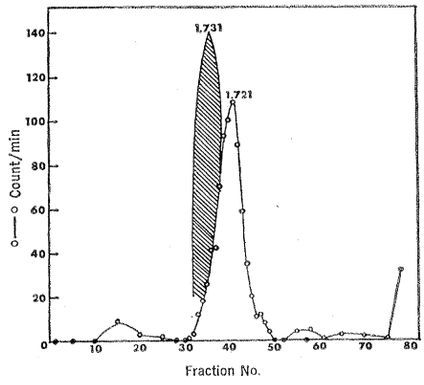

Fig. 2. Rebanding of dense fractions of BrU-labeled DNA from Fig. 1B. Fractions 43 and 44 of Fig. 1B were combined and centrifuged under conditions identical with those given in Fig. 1. The shaded curve represents *Micrococcus lysodeikticus* DNA used as a density marker.

the radioactivity curve as compared with the O.D. curve would be consistent with such heterogeneity. Thus, if fractions corresponding to the denser regions of the radioactivity curve in Fig. 1B were rerun in CsCl, the DNA might then be expected to band at a density corresponding to a thymine replacement value of considerably greater than 15 percent.

Accordingly, fractions 43 and 44 were combined and recentrifuged in CsCl. *Micrococcus lysodeikticus* DNA was added to serve as a density marker since there was insufficient mitochondrial DNA to be detected spectrophotometrically. The results in Fig. 2 show that the DNA rebands at a density of 1.721, indicating a density shift from normal mitochondrial DNA of 0.021 g/cm^3, more than twice that seen previously. This value corresponds to a 33 percent replacement of thymine in these molecules. As a control, DNA fractions from the denser side of the TTP incorporation curve (Fig. 1A, fractions 47 and 48, average density 1.711) were also rerun, and this DNA returned to a position (density = 1.704) close to that of normal mitochondrial DNA (density 1.700). Thus, the extensive synthesis yielding the population of heavy molecules further strengthens the view that the process occurring is replication.

The value of 33 percent replacement was computed on the assumption that the DNA was bifiliarly substituted. If mitochondrial DNA replicates semiconservatively, as is suggested by in vivo experiments (*8*), and if its half-life is somewhere between 4 and 10 days (*20*), then in the 90-minute incubation period it is unlikely that any more than a single strand of any DNA duplex would have replicated. Thus, the value of 33 percent would be equivalent to 66 percent replacement of the thymine if, in fact, the DNA molecules were unifiliarly substituted. This would mean that, in some DNA duplex molecules, almost an entire strand had been synthesized during the incubation period. (The computed value of 66 percent would be valid as an average value irrespective of whether or not the thymine contents of both strands are equal.)

Inherent in the thymine-replacement calculations is the assumption that the BrU-labeled DNA had not been extensively fragmented during the incubation or isolation procedures. Such fragmentation could lead to the appearance of pieces of highly dense DNA with consequently elevated values for BrU content, giving a misleading impression that the intact DNA molecule was as heavily substituted. Ultraviolet radiation of BrU-labeled DNA causes single-strand breaks and could also cause some fragmentation (*21*). However, care was taken to avoid exposure of the DNA to this region of the spectrum; all fluorescent lamps in the laboratory are covered with plastic shields opaque to short and long wavelengths of ultraviolet light, and direct sunlight does not enter the laboratory. Nevertheless, sedimentation velocity studies of the dense DNA are in progress.

The in vivo studies on the turnover rate of mitochondrial DNA (*20*) and on its mode of replication (*8*) do not provide evidence that this DNA is synthesized in the mitochondrion itself. It could, like cytochrome c (*22–24*), malic dehydrogenase (*23*), and probably many other mitochondrial components (*23, 25*) be synthesized elsewhere in the cell and be transported to the mitochondrion after synthesis. The experiments reported here, using isolated mitochondria to study DNA synthesis, provide strong evidence that the rat liver mitochondrion can replicate its own DNA. Confirmation of this view could come from examination of DNA fragments produced by shearing and of the individual strands of denatured DNA. The experiments reported here do not exclude the possibility that DNA repair processes may also occur in mitochondria.

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Monocular Compared to Binocular Depth Perception in Human Infants

Abstract. *Human infants were made temporarily monocular with an eye patch. They performed like bginocular infants on the visual cliff at visual depths of 25 centimeters or more. At a visual depth of 12.7 centimeters infants younger than 9 months of age revealed a monocular weakness by turning toward the uncovered eye.*

Monocular perception of depth has been shown by a number of investigators. Of these, the classical investigations are those of Canella (*1*) who found that monocularization produced little behavioral disturbance in many animals, including predators whose lives depend upon accurate distance perception. Recently, the visual cliff has been used to demonstrate monocular depth perception in such animals as chicks, ducklings, and rats (*2*).

Nevertheless, monocular depth perception in human infants has not been adequately investigated. Only one study of a monocular infant—who had had little experience with binocular depth perception—has been reported (*3*). Normal human infants must be more intensively studied under a variety of conditions to understand strengths and limitations of monocular depth perception. I now describe observations on human infants made temporarily monocular by an elastic eye patch. Monocular as compared to binocular depth perception was studied by the use of several visual depths and stimulus conditions.

The visual cliff for human infants (Fig. 1) consists of an enclosed box (2.44 m in length, 1.52 m in width, and 1 m in height) topped with 1.6-cm Herculite glass (*4*). A center board bisects the glass into two equal segments. Directly under the glass of one side (the shallow side) a textured pattern is placed; at some distance below the glass on the other ("deep") side of the visual cliff another visual surface is placed. The 1.52-m center board was 35.3 cm wide at the end, where the infant was placed, and it tapered to a width of 7.6 cm at the other end. The inside of the apparatus was painted a homogeneous gray. A light meter reading of about 14.3 lu/m^2 was secured by measuring light directly reflected from the patterns. The illumination source was a homogeneous white ceiling with the light diffused through white cotton cloth. Curtains surrounded the apparatus to present a relatively standard plain view of the surrounding environment.

Monocular data were collected gradually over a 4-year period. The distances under the glass for the deep pattern were 1 m, 50 cm, 25 cm, and 12.7 cm. The shallow pattern was usually 1.9-cm red and white checks, but 0.6-cm checks were occasionally used. The deep side contained 1.9-cm or 7.6-cm red and white checks, or a nontextured gray.

The procedure offered the infant a "choice" of the shallow or the deep sides of the visual cliff (Fig. 1). The infant was placed on the wide end of the center board and was called by the mother from the narrow end. To reach the mother the infant almost always left the center board and crawled to her over either the shallow or the deep side (a few straddled).

Binocular infants responded similarly to all of the patterns noted above when the mother offered them such a "choice"; they averaged 96 percent choice of the shallow side with visual depths of 25 cm or more (*4*), and 78 percent choice of the shallow side with the visual depth of 12.7 cm (*5*).

The "choice" constituted the first test trial. After this, infants were called by the mother from both the shallow and the deep sides of the cliff, and were actually placed on the glass and called by her from the shallow end. For the second test trial some of the infants were made monocular at the end of the session by the use of a regular commercial eye patch (with an elastic band to hold it in place). Latencies on the first test trial were often long, with a substantial minority over 1 minute, and the eye patch might not be accepted at all if it was given first. Three classes of infants were represented on the second test trial: (i) those who accepted the eye patch (monocular), (ii) those who took it off (binocular), and (iii) those given the last trial without trying the eye patch at all (binocular). The last group was studied to determine whether infants who accept or reject the eye patch differed from each other in some way (*6*).

Because monocular infants with visual depths of 25 cm (10 inches) or more responded similarly and stimulus patterns seemed to make no difference, these data are presented first. The results with the 12.7 cm (5 inches) of depth follow.

Table 1 presents the data for babies