Short- and Long-Lived Messenger RNA in Embryonic Chick Lens

Abstract. *Cells of the embryonic chick lens pass through a definite sequence of macromolecular synthesis as they differentiate and migrate from one zone of the organ to another. Autoradiographic studies of RNA synthesis and protein synthesis show that long- and short-lived messenger RNA's are both present in the lens.*

A previous study of the disappearance of protein synthesis on polyribosomes after treatment with actinomycin D has indicated that messenger RNA's (mRNA's) of different life spans coexist in the developing chick lens (*1*). We now offer further evidence in support of that finding and indicate how they are segregated spatially.

At about 8 days of incubation, the cells of the main body of the lens which will make up the core of the adult organ cease to enlarge (*2, 3*). A cortex of new cells is added to the body as cells from a germinal zone of epithelium are displaced posteriorly, pass through an intermediate zone called the annular pad, and finally are transformed into elongated fiber cells which are wrapped in nearly concentric layers around the core (Fig. 1*a*). Thus there exists a gradient of cells that become progressively more differentiated as they move from the proliferating epithelium into the fibrous body.

Synthesis of DNA, RNA, and protein in the various parts of the 12-day-old chick lens were studied autoradiographically in lenses labeled in vitro with protein or polynucleotide precursors. The life span of mRNA was examined by observing the disappearance of protein synthesis after treating 12-day-old lenses with enough actinomycin D (30 μg/ml) to stop 99 percent of total RNA synthesis within 2 hours (*1*).

The pattern of C^{14}-thymidine uptake into DNA after a 3-hour treatment period in vitro is shown in Fig. 1*a*. Digestion with deoxyribonuclease of control sections completely removed this radioactivity. Synthesis of DNA is localized in the epithelium anterior to the annulus, a finding consistent with earlier histological observations of cell division in embryonic chick lenses (*3*). The separation of cells synthesizing DNA from those not actively engaged in DNA synthesis was quite sharp in all lenses studied. Autoradiography of rabbit lens epithelium labeled in vivo has suggested that, in an adult lens, thymidine incorporation is restricted to a ring of germinative cells just anterior to the annulus (*4*). The effect of incubation in vitro on chick lenses could be responsible for the difference seen here.

The pattern of C^{14}-uridine uptake in lenses labeled for 3 hours in vitro is shown in Fig. 1*b*. The cells most active in RNA synthesis are located in the epithelium, but a line of incorporation extends into the body from either side; it is superimposed over the nuclei of the concentrically arranged fiber cells. Digestion of fixed sections with ribonuclease completely removed radioactivity due to C^{14}-uridine incorporation. RNA synthesis appears to decrease in cells as they enter the body, and, toward the core, becomes undetectable by this method, even though at 12 days a few scattered nuclei are still present in the core. Control lenses which were cut in half and then labeled with C^{14}-uridine showed essentially the same pattern of incorporation as shown in Fig. 1*b*.

The flow of RNA from the nucleus into the cytoplasm seems to decrease as cells enter the body region. However, higher-resolution autoradiography will be necessary to establish this point. Addition of actinomycin D simultaneously with the isotope totally inhibited C^{14}-uridine incorporation, indicating that RNA synthesis is terminated immediately by this antibiotic.

The pattern of C^{14}-leucine incorporation into 12-day-old lenses after a 1-hour exposure to isotope in vitro is shown in Fig. 1*c*. All sections labeled with C^{14}-leucine were extracted with 5 percent trichloroacetic acid at 90°C for 30 minutes. The greatest concentration of radioactivity is again observed in the epithelium; but grains are also seen over the entire body, decreasing in concentration from the epithelium and annulus toward the core. Virtually no gradient was observed between the posterior surface of the lens and the

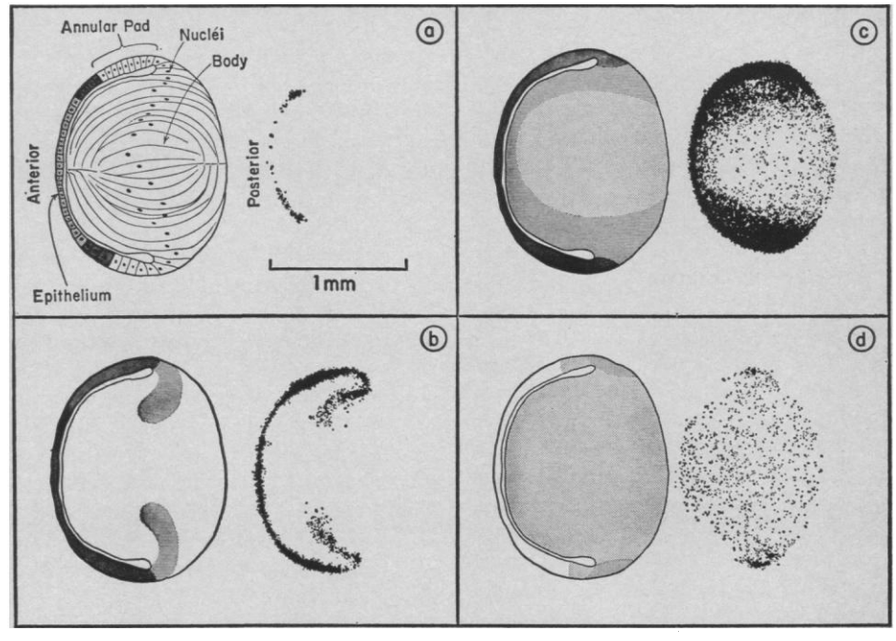

Fig. 1. Autoradiographs of 12-day chick lenses. Lenses were incubated in vitro in 2 ml of Charity Weymouth Medium containing penicillin (0.4 mg), streptomycin (0.25 mg), phenol red indicator, and 10 μc of radioactive precursor (New England Nuclear Corp.) as indicated below. Fixation was in Susa's fluid (*7*). Paraffin sections, 10 μ thickness, mounted on glass slides, were exposed to Kodak No-Screen medical x-ray film for 1 to 2 days. The diagrammatic sketches on the left of each autoradiograph represent histological sections showing the parts of the lens. The body consists of a central core of cells surrounded by a cortex. The intensities of stippling over the parts correspond to the distribution of grains in the autoradiograms. (*a*) Pattern of incorporation of C^{14}-thymidine. Radioactivity is restricted to the epithelium and is concentrated in a ring of cells anterior to the annular pad; (*b*) C^{14}-uridine is incorporated by cells in the epithelium and by those in the cortex of the body, but not by cells in the core. In the body, incorporation is restricted to the nuclear regions of the cells; (*c*) C^{14}-leucine is incorporated in greatest degree by cells of the epithelium, to a lesser extent by cells of the cortex of the body, and least by cells of the core. Cells which have just entered the cortex may have as much label as those in the epithelium; (*d*) C^{14}-leucine after 8 hours in actinomycin D is incorporated uniformly throughout the body but not at all in the epithelium. Cells which have just entered the body appear to have taken up more leucine than other cells.

core. The fact that leucine is incorporated in the core of the lens, a region where RNA synthesis is undetectable, implies that a relatively stable species of mRNA is present. Since the reservation must be made that very small amounts of RNA synthesis would not be detected by the autoradiographic technique used, the following experiment was performed to examine messenger stability more closely. Two groups of lenses were incubated, one in Charity Weymouth Medium and the other in the medium to which actinomycin D (30 μg/ml) was added for periods up to 10 hours. At intervals lenses were taken from each group and treated for 1 hour with C^{14}-leucine. Autoradiography of lenses treated with the antibiotic reveals that the heavy incorporation originally seen in the epithelium progressively disappears whereas the core remains unaffected. The control lenses incubated without the antibiotic showed no change.

After 8 hours in actinomycin D (Fig. 1*d*) the rate of incorporation in the cortex and part of the annulus had decreased to the level of that in the core with the exception of a small, highly radioactive ring around the equator. Virtually all incorporation had ceased in the epithelium. This pattern suggests that a stable mRNA exists throughout the body and part of the annulus whereas an unstable mRNA is present in the epithelium. Thus both stable and unstable mRNA's may coexist in cells of the cortex and part of the annulus. Observations on decrease of specific activities of electrophoretically separated proteins from various parts of the lens support these interpretations (*6*).

Our data indicate that the lens contains mRNA's of different half-lives, some relatively long and others short. Higher resolution and more quantitative autoradiography will be required to show whether both types of message coexist in some cells.

In control lenses there is almost no gradient of incorporation from the posterior surface of the lens to the core, and the C^{14}-leucine grains are evenly distributed after actinomycin treatment. This fact suggests that the pattern of C^{14}-leucine incorporation is not an artifact of diffusion. Since bisected lenses incorporated C^{14}-uridine in essentially the same pattern as whole ones, the pattern of RNA synthesis can also not be attributed to diffusion.

These data imply that lens cells pass through a definite sequence of macromolecular syntheses as they differentiate and migrate from one zone of the lens into another; DNA is synthesized exclusively in a region of the epithelium anterior to the annulus. In that zone, cells also make RNA and synthesize protein on the mRNA that appears to have a short half-life. In part of the annulus and throughout the entire body region, cells make protein on long-lived mRNA's. Possibly some cells of the cortex and annulus contain both short- and long-lived mRNA. The stable mRNA is present in greatest concentration in a ring of cells around the equator of the lens. RNA synthesis in the cortex decreases gradually toward the core, and its flow into the cytoplasm may be reduced. Cells of the core make little if any RNA but continue to synthesize protein for some time on stable mRNA.

A number of regulatory events must occur as the foregoing sequence is executed. (i) Genes concerned with DNA synthesis are repressed as cells move into the annulus. (ii) Either mRNA which was formerly unstable is stabilized, or genes concerned with the synthesis of stable mRNA's are activated when cells move through the annulus into the cortex. (iii) RNA synthesis is terminated in cells of the core.

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References and Notes

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8. We thank Diane Major for help in sectioning lenses. Supported by a grant from NSF (GB614). R.R. is an NSF predoctoral fellow.

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Catalase: Kinetics of Photooxidation

Abstract. *The kinetics of photooxidation of catalase is a four-step consecutive reaction, if each of the four porphyrin moieties acts independently. The rate of enzyme inactivation is a first-order reaction, resulting from destruction of a single porphyrin. The kinetics of absorbancy is more complex, depending upon the absorption probabilities of each of the microspecies. With equal probabilities, the log of the sum of the normalized absorptivities is a linear function of time.*

Catalase is a heme ezyme, believed to consist of four porphyrin moieties residing within identical or nearly identical subunits (*1*). The intact aggregate is required for enzyme activity; consequently, the destruction by photooxidation of only one of the porphyrins would suffice for complete inhibition, even though three-fourths of the absorbancy in the Soret band region (405 nm) remained. The kinetics of photooxidative enzyme loss will therefore be different from that of absorbancy loss (*2*).

The following model appears to provide the common kinetics for the two phenomena. If each of the porphyrin-containing monomers in the aggregate absorbs light and is destroyed independently, then at any time, t_i, after t_0, the population will contain four microspecies, with 4, 3, 2, or 1 heme residues per molecule (Fig. 1). The rate of the photochemical reaction will be a function of the concentration, C_i, of the absorbing species, of its absorptivity, k, of the light flux, and of the possible differential receptivity of each of the hemes, K_i, in the various monomers within the aggregate. For any given constant light flux, and in an optically thin solution, the set of differential equations describing the transformation of the porphotetramer into the porphotrimer, and so forth, is as follows, the group being designated Eq. 1.

$$\begin{aligned}
\frac{dC_4}{dt} &= -4\,kK_4C_4 \\
\frac{dC_3}{dt} &= 4\,kK_4C_4 - 3\,kK_3C_3 \\
\frac{dC_2}{dt} &= 3\,kK_3C_3 - 2\,kK_2C_2 \\
\frac{dC_1}{dt} &= 2\,kK_2C_2 - kK_1C_1
\end{aligned} \qquad (1)$$

The foregoing equations are of linear form and may be integrated by standard methods to yield the following set, which is designated Eq. 2 (p. 73).

The k's are a function of the wave length, λ. Since k is known for the enzyme, K_4 may be calculated from its kinetics. The absorbancy is the sum of contributions from each of the microspecies (Eq. 5, below), and it should be possible, in principle, to calculate the K_i's independently from the experimental data. However, for any given $k_\lambda = k$, if we assume the K_i's are identical, $kK = R$, where R is a constant. With these conditions and that of $C_0 = 1$, the above equa-