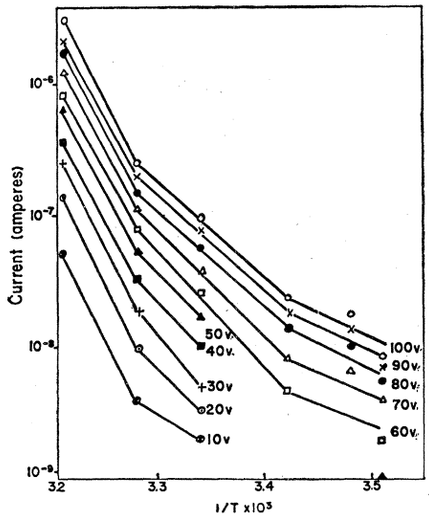

Fig. 2. Current as a function of reciprocal temperature for different voltages.

ergies, which preclude their representing different energy levels. Second, the observed activation energies are independent of voltage despite the current's rough dependence upon the square of the voltage; the curves for different voltages (Fig. 2) have the same shape. (There is evidence that moisture plays some role. When the sample was tested after an interval of some weeks, its dark current had decreased by about an order of magnitude, presumably because of further drying.)

Figure 3 shows the photocurrent, which is indistinguishable from the dark current except at lower temperatures. At 7°C, the current is doubled by 5 mw of light from 4200 to 5000 Å falling on the sample (between the electrodes). The increase could be produced by ultraviolet, blue, and green light. However, red light of the same intensity as that of each of the other wavelength regions failed to produce an increase over the dark current, indicating that the observed increase is indeed a photocurrent, and not the effect of heating (*3*). Lowering the temperature further did not result in a further increase in photocurrent. Instead, the current leveled off, and declined at temperatures below 4°C. (Some light could reach the electrodes, which might lead to possible artifacts due to light sensitivity of the silver. However, our discussion pertains to the dark current rather than the photocurrent.)

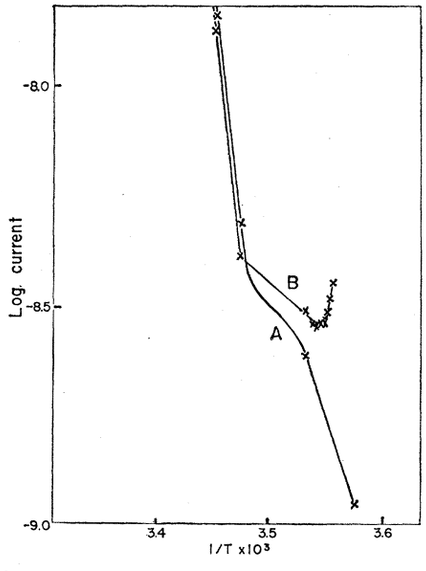

Fig. 3. Log current as a function of reciprocal temperature at 1 volt in the dark (curve A) and under illumination by 5 mw of light from 4200 to 5000 Å (curve B).

It is difficult to reconcile activation energies of 1.47 to 2.94 ev with currents of 10^{-8} to 10^{-6} amp/cm^2 with a semiconductor model. This is a difficulty that applies to many studies of biological materials. For ΔE equal to 1.47 ev the exponential factor equals 10^{-26}. From the expression commonly employed for i_0

$$i_0 = 2n_0 e\mu\epsilon$$

where n_0 is the carrier density, e is the electronic charge, μ is the mobility, and ϵ is the field strength, and substituting the largest feasible values for n_0 and μ, 10^{22}/cm^3 and 1 cm^2/volt sec (although mobilities of 1000 have been postulated for tunneling or similar processes) (*1*), we find that the magnitude of i_0 is 10^5, yielding a current of 10^{-21} amp/cm^2. Kallmann and Pope (*4*) have argued that the above expression applies only when charges originate at the surface, and that the appropriate expression for the maximum current density thermally produced in the bulk material is

$$i = n_0 e\delta d e^{-2\Delta E/KT}$$

where ΔE is the measured quantity and equals half the energy gap, d is the sample thickness, and δ is the collision factor. If δ equals 10^{12} sec^{-1}, the preexponential factor is 10^{15}, and the exponential factor 10^{-52}, yielding a current of 10^{-37} amp/cm^2, a strong indication, since the measured currents are much greater, that we are dealing with surface effects.

One alternative to using standard semiconductor theory would be to conclude that the measured activation energy is not that required to deliver charges, but relates, perhaps, to the resistance of the sample. One might envisage charge production at the electrode-sample interface with low activation energy (*4*), combined with transport through the bulk with high activation energy (*5*) (precluding transport through a conduction band). The sample might be "porous," with the "pores" or channels more conductive than the material and becoming increasingly conductive with temperature. The activation energy is then a measure of the effectiveness of the channels in transporting charges from the surface. Like normal semiconductor electron and hole conduction, protonic conduction is ruled out because the measured currents are too high.

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Histone Synthesis in vitro by Cytoplasmic Microsomes from HeLa Cells

Abstract. *HeLa cell microsomes incorporate labeled amino acids in vitro into acid-soluble proteins which have the same electrophoretic mobility as histones isolated from the purified HeLa cell nuclei. The capacity to synthesize histones in vitro is dependent on deoxyribonucleic acid synthesis in the cells from which the microsomal fraction is prepared.*

The accumulative synthesis of histones in mammalian cells is temporally coupled to the synthesis of DNA during chromosome replication (*1–3*). This relationship, as well as the close physical combination of histones with the DNA, suggests that specific DNA-histone as-

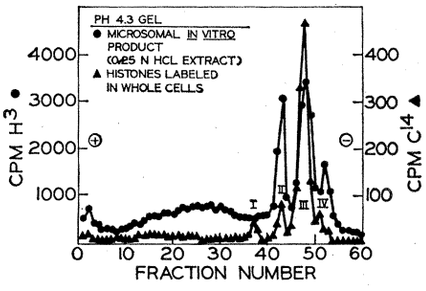

Fig. 1. Polyacrylamide gel-electrophoretic pattern of acid-soluble, ^{3}H-labeled polypeptides synthesized on microsomes in vitro and a ^{14}C-labeled histone marker. Electrophoresis was performed in 10 percent gels for 4 hours at 5 ma per gel, *p*H 4.3, and room temperature.

sociations that function in the control of gene expression (*4*) and in the stabilization of a cell's differentiated state may be formed during nuclear replication.

To explore this relationship we have attempted to synthesize histones in cell-free systems derived from synchronized cultures of HeLa cells and to study the nature of the coupling between histone synthesis and DNA replication.

We developed a nuclear system which continued the synthesis of nuclear proteins in vitro (*5*). While many proteins were labeled, we were surprised to find that the nuclei were unable to synthesize histones. The biosynthesis of histones occurred in the microsome fraction isolated from the cytoplasm of HeLa cells that were in the process of nuclear replication. We now describe the cytoplasmic system for the continuation of histone synthesis in vitro and the dependence of this activity on the concurrence of DNA synthesis in the nuclei of the cells from which the microsomal system was prepared. The demonstration that histone synthesis occurs in the cytoplasm is in agreement with the in vivo findings of Robbins and Borun (*3*) who first reported the association of histone-like polypeptides with a distinct class of cytoplasmic polysomes which also containing a distinctive species of rapidly labeling RNA (*6*).

For these experiments HeLa cells were grown in suspension cultures in a modified Eagle's medium (*7*). To synchronize the cells for DNA synthesis, the cultures were treated with $10^{-6}M$ amethopterin and $5 \times 10^{-5}M$ adenosine for 16 hours. A synchronous wave of DNA synthesis was then achieved by addition of thymidine (5 μg/10^6 cells) to reverse the induced thymidineless state. Cells were lysed with the aid of a Dounce homogenizer in a hypotonic medium consisting of $0.01M$ tris (hydroxymethyl) amino methane-hydrochloride (tris-HCl) (*p*H 7.5), $0.0015M$ $MgCl_2$, and $0.005M$ 2-mercaptoethanol. After the nuclei and large particulates were removed by centrifugation at 20,000*g*, the microsome fraction was sedimented at 110,000*g* for 60 minutes. This fraction was suspended in incubation medium [$0.05M$ tris-HCl (*p*H 7.6), $0.005M$ $MgCl_2$, $0.05M$ NH_4Cl, and $0.005M$ 2-mercaptoethanol], and large aggregates were removed by centrifugation at 12,000*g* for 5 minutes. The middle third of the 110,000*g* supernatant was used as the soluble enzyme and transfer RNA support in the incubations in vitro. The incubation mixture contained, in a final volume of 1 ml, 50 μmole of tris-HCl, 5 μmole of $MgCl_2$, 50 μmole of NH_4Cl, 5 μmole of 2-mercaptoethanol, 1 μmole of adenosine triphosphate (ATP), 0.2 μmole of guanosine triphosphate (GTP), 10 μmole of phosphoenol pyruvic acid, 4 μg of pyruvate kinase, 2 nmole of L-(^{3}H)-leucine (specific activity, 40 c/mmole), 1.35 nmole of ^{3}H-lysine, (L-form, specific activity, 45 c/mmole), 4.2 nmole of ^{3}H-arginine (L-form, specific activity, 1.175 c/mmole), microsomes (1.5 to 2 mg of protein), and 110,000*g* supernatant (0.5 to 0.7 mg of protein).

The cell-free amino acid–incorporating system from HeLa cell microsomes used in this study required 5 mM $MgCl_2$ and 50 mM NH_4Cl for optimal activity at *p*H 7.6. An energy source was also needed as well as the addition of 110,000*g* supernatant; the latter yielded a three- to fourfold increase of the protein-synthesizing activity. Puromycin ($1.5 \times 10^{-4}M$) and ribonuclease A (5 μg/ml) inhibited the amino acid incorporation by more than 95 percent.

In a typical experiment the incorporation was stopped after 30 minutes by the addition of 50 μg/ml of ribonuclease A. The incubations were continued for 10 minutes, and the acid-soluble proteins were then extracted from the incubation mixture with $0.25N$ HCl. After exhaustive dialysis against $0.025N$ HCl, the acid-extractable proteins were precipitated with ten volumes of acetone at −10°C and dried in a vacuum desiccator. To resolve the different histone species, the ^{3}H-labeled acid-soluble protein fraction labeled in vitro was dissolved in $10M$ urea with

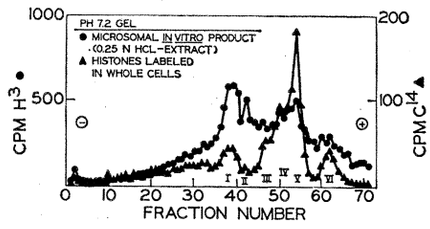

Fig. 2. Polyacrylamide gel-electrophoretic pattern at *p*H 7.2. The proteins were treated as described and subjected to electrophoresis for 14 hours at 5 ma per gel and room temperature in 10 percent gels according to Shapiro *et al.* (*9*). The gels contained $6M$ urea.

marker histones, isolated from nuclei labeled in vivo with ^{14}C-leucine, and separated electrophoretically in 10 percent polyacrylamide gels at *p*H 4.3 (*8*). The histones which were used as markers were derived from synchronized cells labeled in vivo with ^{14}C-leucine (specific activity, 175 mc/mmole) for 6 hours during DNA synthesis period; the histones were extracted from highly purified nuclei (*5*).

A typical electrophoretic pattern of the acid-soluble proteins synthesized on microsomes that were isolated from cells during DNA synthesis is shown in Fig. 1. The two most prominent peaks of the in vitro product coincide exactly with the position of two of the four main radioactive peaks of HeLa cell histones. When the same proteins were dialyzed for 20 hours against $0.1M$ sodium phosphate buffer (*p*H 7.2)

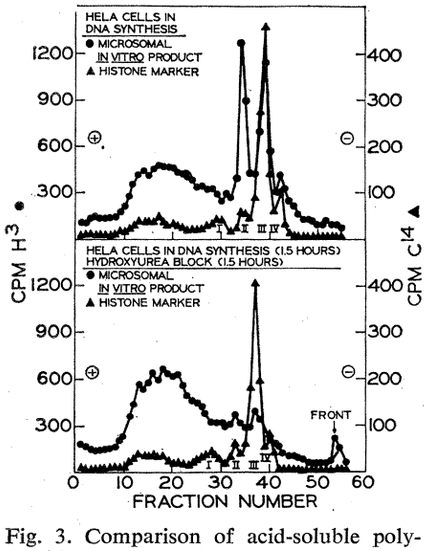

Fig. 3. Comparison of acid-soluble polypeptides synthesized in vitro on microsomes isolated from cells in DNA synthesis (top) and cells blocked with $2 \times 10^{-3}M$ hydroxyurea (bottom). Electrophoresis was performed at *p*H 4.3.

containing 6*M* urea, 0.14*M* 2-mercaptoethanol, and 0.1 percent sodium dodecyl sulfate (SDS) and subjected to electrophoresis at *p*H 7.2, the marker histones separated into six major radioactive peaks; four of these marker peaks coincide with the ^{3}H-labeled peaks from the proteins synthesized in vitro (Fig. 2). Whereas the separation in the electrophoretic system at *p*H 4.3 is based mainly on charge, the separation at *p*H 7.2 is primarily based on molecular size (*9*).

To further establish the identity of the histones synthesized in vitro in the microsomal system with the marker histones isolated from nuclei labeled in vivo, the histone bands II and III from the *p*H 4.3 gels were isolated and again subjected to electrophoresis at *p*H 4.3 and 7.2. For this purpose the bands were first localized by rapid staining with tetrabromophenolphthalein ethyl ester (*2*), and the protein in the bands was extracted with 0.025*N* HCl. The ^{3}H-labeled proteins and the ^{14}C-labeled histone bands II and III appeared as single peaks in exactly the same position upon electrophoresis again at *p*H 4.3. At *p*H 7.2 band II as well as band III split into two stainable subbands. However, in each case the ^{3}H- and the ^{14}C-labeled proteins coincide again after reelectrophoresis under these conditions.

Our earlier studies (*2*), confirmed by Robbins and Borun (*3*), showed the close temporal coupling in the accumulative synthesis of DNA and histones. With hydroxyurea to block DNA synthesis (*10*), it was demonstrated that this relationship holds as well for the synthesis of histones in vitro. For this purpose synchronized HeLa cells were allowed to synthesize DNA for 90 minutes; DNA synthesis was then blocked in representative cultures for a further period of 90 minutes by the addition of $2 \times 10^{-3}M$ hydroxyurea. The microsomal system isolated from cells in which DNA synthesis had been blocked for 90 minutes exhibited only a marginal labeling of the histone, compared to the microsomes isolated from cells which were actively synthesizing DNA in vivo for 90 or 180 minutes (Fig. 3). The capacity for histone synthesis was not altered when the microsomes from cells in DNA synthesis were incubated in the presence of soluble supernatant fraction from hydroxyurea-blocked cells and vice versa. This indicates that the microsomal, rather than the supernatant fraction was modified by the blockade of DNA synthesis with hydroxyurea in the living cell.

Thus HeLa cell microsomes isolated from the cytoplasm of cells which are in process of DNA synthesis can synthesize histone proteins in vitro. These proteins are electrophoretically identical with marker histones isolated from nuclei labeled in vivo. The dependence of this cytoplasmic system on the synthesis in vivo of DNA also provides a means for exploring the nature of the coupling between DNA replication and histone synthesis.

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Microbody-Like Organelles in Leaf Cells

Abstract. *An organelle approximately 0.5 to 1.5 microns in diameter, limited by a single membrane, occurs abundantly in the chlorophyllous cells of leaves of several dicotyledonous and monocotyledonous plants. Its finely granular matrix frequently contains crystalline, fibrous, or amorphous inclusions. It is frequently appressed to a chloroplast or squeezed between chloroplasts so that its limiting membrane is in extensive contact with the outer membranes of the chloroplast envelopes. The organelle is probably identical with recently isolated leaf particles that contain enzymes involved in the metabolism of glycolate, a chloroplast product; it is interpreted as a form of plant microbody.*

We have observed that an organelle resembling a plant microbody (*1*) occurs abundantly in green leaf tissues. The organelle is a common component of the mesophyll cells of several species examined (Figs. 1 and 2) and frequently occurs appressed to chloroplasts.

We observed the organelle, temporarily termed a "cytosome," in material fixed in glutaraldehyde and osmium tetroxide and processed for electron microscopy by standard procedures (*1*). Sections were stained with uranyl acetate and lead citrate. Although the cytosome varies in morphology somewhat in different species, it characteristically has a diameter between 0.5 and 1.5 μ, a rounded to irregular shape, and a matrix of moderate electron opacity enclosed by a single membrane. The finely granular, homogeneous matrix frequently contains dense crystalline, fibrous, or amorphous inclusions.

Leaf tissues of several dicotyledonous and monocotyledonous plants were examined, including bean (*Phaseolus vulgaris*), *Petunia* sp., *Torenia fournieri*, snapdragon (*Antirrhinum majus*), wheat (*Triticum vulgare*), and barley (*Hordeum vulgare*). Special emphasis was placed on mature leaves of tobacco (*Nicotiana tabacum*, var. "Wisconsin 38") and on both young and mature leaves of timothy (*Phleum pratense*). In all these species, cytosomes similar to those of tobacco and timothy were observed in many cells of both nonvascular and vascular tissues. They were particularly large and numerous in cells containing chloroplasts. In seedlings of a few other species (for example, corn), cytosomes were present but were smaller and relatively scarce. We have not determined the nature of cytosomes in mature leaves of these species.

Mature mesophyll cells have a relatively simple structure in which a layer of cytoplasm rich in free ribosomes surrounds a large central vacuole. Cisternae of the endoplasmic reticulum (ER) are present in moderate amount, but dictyosomes are relatively scarce. Chloroplasts are commonly aligned in rows in a single layer near the walls. Mitochondria are abundant around the chloroplasts. The cytosomes are similarly distributed but show a greater tendency to lie wedged tightly between adjacent chloroplasts. The relative number of cytosomes varies with species