the electrodes. There are several important points about the siphon that should be mentioned:

1) The dielectric siphon is self-priming and can be controlled remotely by the voltage V.

2) Once V is raised beyond the threshold, the fluid cross section remains essentially constant, and so the flow rate of the parallel-plate device (Fig. 1) is independent of voltage. (Other dielectric siphons are envisioned, in which continuous flow control would be achieved.)

3) The electrical polarization force present does *not* pump the dielectric liquid, but merely provides an orientation configuration with fluid communication between the two reservoirs. As with any true siphon, gravity does the pumping.

To test the theoretical model for the dielectric siphon, Perry measured indirectly the flow rate as a function of time for various siphon parameters (*8*). If we assume viscous laminar (Poiseuille) flow between the electrode plates and neglect inlet and outlet flow losses, the theoretical rate of volume flow Q in the siphon is a decaying exponential function of time t with a time constant τ^*:

$$Q(t) = Q_0 e^{-t/\tau^*} \quad (2)$$

$$\tau^* = \left(\frac{A_1 A_2}{A_1 + A_2}\right) \frac{12\mu L}{gs^3 w\rho}, \; A_1 \simeq A_2 \quad (3)$$

where Q_0 is the theoretical rate of volume flow at time $t=0$, A_1 and A_2 are the cross-sectional areas of the two reservoirs, μ is the dynamic liquid viscosity, L is the working length of the siphon, and w is the electrode width (for the experiment reported h was approximately 2.5 to 5 cm). Plots of the experimental values of Q versus t on semilogarithmic paper are linear, an indication of the validity of Eq. 2. Figure 3 shows a plot of the time constants taken from numerous experimental measurements, with a theoretical curve from Eq. 3. The results are independent of the influence of the applied voltage V.

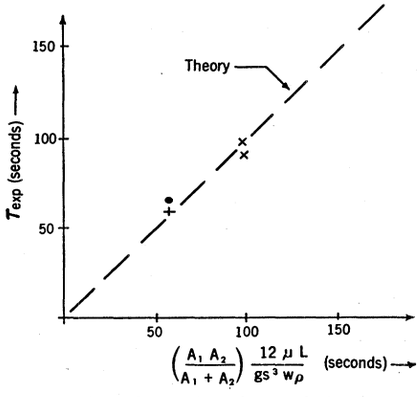

Fig. 3. Plot of the experimental time constant τ_{exp} versus the theoretical time constant τ^*. Root-mean-square voltages are as follows: ×, 19 kv; •, 30 kv; +, 34 kv.

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9. The work reported here was performed at the Massachusetts Institute of Technology, Cambridge. This work is based in part on a thesis entitled "Dielectrophoretic siphons" submitted (by M.P.P.) to the Department of Electrical Engineering at the Massachusetts Institute of Technology in partial fulfillment of the requirements for the degree of Bachelor of Science. We thank Prof. J. G. Trump of the High Voltage Research Laboratory for the loan of a pressure vessel. Supported by NASA grant NGL-22-009-014.

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Nuclear Acidic Protein Changes during Differentiation in Physarum polycephalum

Abstract. *A class of acidic nuclear phosphoproteins has been isolated throughout the mitotic cycle and at two points during differentiation in the slime mold* Physarum polycephalum. *The electrophoretic profiles of these proteins are reproducible and unchanging throughout the mitotic cycle, but reproducible changes occur during differentiation. The proteins are rapidly synthesized after mitosis, and their molecular weights range from 34,000 to 88,000. The proteins rapidly incorporate [^{32}P]orthophosphate, and the content of alkali-labile phosphate increases 20 percent during the period after DNA synthesis. The proteins comprise 6.5 percent by dry weight of nuclear material while the DNA comprises about 5.5 percent. These acidic nuclear proteins may have a role in control of gene activity.*

The regular distribution of nuclear histones throughout many types of cells (*1*, *2*), together with the unchanging nature of histones during tissue differentiation (*3*) and the apparently nonspecific nature of histone inhibition of DNA-directed RNA synthesis (*4*), complicates any model attempting to explain the mechanism of nuclear histones in controlling specific gene expression. In contrast the heterogeneity (*5*, *6*) and tissue specificity (*6*) of certain acidic nuclear proteins suggests that these acidic proteins may in fact possess the required specificity and variety of types necessary for control of gene activity. Whereas the studies of others (*4–11*) imply a gene regulatory function for the acidic nuclear proteins, more direct evidence, such as major changes in the electrophoretic profiles of these proteins in differentiating tissue, has not been reported.

This report is concerned with the characterization of a specific phenol-soluble fraction of acidic nuclear proteins in *Physarum polycephalum*. The procedure for protein isolation was adapted from Teng *et al.* (*6*). Proteins isolated from nuclei through phenol solubilization have been shown to be synthesized specifically in response to hormones (*7*), and these proteins are heterogeneous in different tis-

Table 1. Amino acid analysis of PSANP fraction of *P. polycephalum*. The values are the average of three analyses on proteins isolated 4 hours after metaphase III (*12*).

Amino acid	Content (moles per 100 moles of total amino acid)
Lysine	7.65
Histidine	2.82
Arginine	5.92
Aspartic acid	10.51
Threonine	5.59
Serine	6.95
Glutamic acid	11.53
Proline	5.19
Glycine	8.60
Alanine	8.27
Valine	6.36
Methionine	1.42
Isoleucine	4.86
Leucine	8.34
Tyrosine	2.65
Phenylalanine	3.46

sues (*6*) and display species-specific interactions with DNA (*6*).

The acidic nuclear proteins solubilized by phenol from rat liver and kidney contain appreciable amounts of esterified phosphate in phosphoserine and phosphothreonine (*6, 8*). Turnover in the phosphates of nuclear phosphoprotein occurs without replacement of serine, and the rate of phosphorylation of certain acidic nuclear proteins increases at times of gene activation (*10*); these results further indicate that the acidic phosphoproteins are involved in control mechanisms. Detailed supportive evidence for the active metabolic role of nuclear phosphoproteins has been reported (*10, 11*).

The multinucleate plasmodium (diploid vegetative growth phase) of the slime mold *Physarum polycephalum* is a highly undifferentiated eukaryotic syncytium, the nuclei of which undergo synchronous mitosis every 8 hours. There is no G_1 period (*12*), and the S period is 3 hours (*13*). The plasmodium cannot be induced by light to sporulate if nutrients are present, and the photosensitive state (a prerequisite for differentiation) develops only after a threshold period of starvation.

Advantage was taken of the natural mitotic synchrony to determine the period in the mitotic cycle when the phenol-soluble acidic nuclear proteins (PSANP) are synthesized and to compare proteins isolated at various points in the mitotic cycle. The ability to control and induce sporulation with light enabled us to correlate changes in the PSANP fraction with the development of the photosensitive state in the plasmodium.

A single isolate of *P. polycephalum* M_3C VII (*14*), resporulated for increased fruiting efficiency, was used exclusively. Previously described procedures for axenic culture of plasmodia (*14, 15*), isolation of nuclei (*16*), and induction of the photosensitive state (*17*) were used.

Nuclear components soluble in saline were removed from isolated nuclei with 0.14*M* NaCl. Proteins soluble in acid were removed with 0.25*N* HCl. Lipids and phospholipids were extracted twice with chloroform-methanol containing 0.2*N* HCl. In the first extraction the proportion of solvents was 1:1, and in the second extraction the proportion was 2:1. The second extraction was followed by a single ether extraction. The residual nuclear pellet was resuspended in 0.1*M* tris(hydroxymethyl)-aminomethane hydrochloride buffer

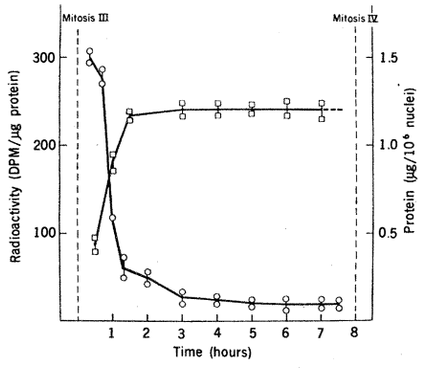

Fig. 1. Incorporation of tritiated amino acids into PSANP and net increase of PSANP. Dashed vertical lines represent metaphase. For each point, 10 percent of the isolated protein was solubilized in scintillation counting fluid and analyzed for disintegrations per minute (*DPM*) by scintillation spectrometry. Protein was determined by the method of Lowry *et al.* (*20*).

(*p*H 8.2) containing 0.01*M* ethylenediaminetetraacetic acid (EDTA) and 0.14*M* 2-mercaptoethanol. This suspension was homogenized twice with equal volumes of phenol that had been saturated with the above buffer. The proteins were prepared for electrophoresis by dialysis against a solution containing 0.01*M* acetic acid and 0.14*M* 2-mercaptoethanol, against 9.0*M* urea buffer solutions, and finally against 0.01*M* phosphate buffer (*p*H 7.2) containing 0.14*M* 2-mercaptoethanol and 0.1 percent sodium dodecyl sulfate (SDS).

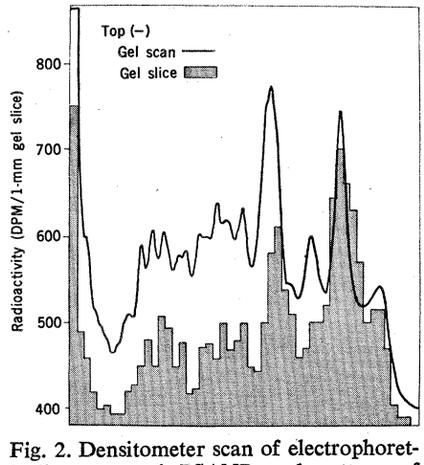

Fig. 2. Densitometer scan of electrophoretically separated PSANP and pattern of radioactivity. The plasmodium was transferred at metaphase III (*12*) to growth medium deficient in amino acids and containing hydrolysate of tritiated protein (0.008 mc/ml), and was incubated for 1 hour. Disintegrations per minute (*DPM*) in individual bands were determined by scintillation spectrometry on 1-mm transverse slices of gels solubilized in 0.5 ml of 30 percent hydrogen peroxide. For electrophoresis procedure see Fig. 3.

For comparison of electrophoretic profiles in polyacrylamide gels during the mitotic cycle, the PSANP fraction was isolated every hour during the S and G_2 periods. For comparison of electrophoretic profiles of the PSANP fraction in the actively growing plasmodia to profiles of fractions present during differentiation, nuclei were isolated from photosensitized plasmodia before illumination and again 8 hours after illumination. The isolated protein fractions were then subjected to coelectrophoresis and specific changes were identified.

The acidic nature of the PSANP fraction is shown by the ratio of acidic to basic amino acid residues of 1.35 (Table 1) and by the anode-directed migration at *p*H 7.0 and 9.0 and cathode-directed migration at *p*H 3.0 of protein fractions solubilized with urea (*18*).

The period in the mitotic cycle when the PSANP fraction is synthesized was determined by the use of 1-hour labeling periods throughout the mitotic cycle and 20-minute labeling periods during the first 80 minutes after metaphase. Cultures were labeled with hydrolysates of tritiated protein in growth medium deficient in amino acids (Fig. 1). To measure incorporation of labeled amino acids into specific proteins, radioactivity in 1-mm gel slices was determined in a scintillation counter (Fig. 2). Net synthesis of PSANP was shown by measurement of PSANP in nuclei isolated at four 30-minute intervals after mitosis and then at 1-hour intervals throughout the remainder of the cell cycle (Fig. 1).

The molecular weight of electrophoretically resolved proteins was determined by coelectrophoresis with protein standards complexed with SDS (Fig. 3). The molecular weights and amino acid composition of acidic nuclear proteins from various rat tissues (*6, 7*) and of PSANP from *P. polycephalum* are very similar, results indicating the specificity of the phenol extraction procedure. However, the electrophoretic profiles of the acidic nuclear proteins from rat tissue are not similar to the PSANP of *P. polycephalum*. The nuclear histones of *P. polycephalum* are similar to those in calf thymus (*2*). In *P. polycephalum*, the ratio of nuclear DNA to the PSANP, like nuclear histones, is almost 1; 6.5 percent by dry weight of nuclear material is PSANP and 5.5 percent is DNA (*19*).

For the G_2 period, the amount of total phosphate and of alkali-labile

phosphate (assayed colorimetrically as the phosphomolybdate complex) in the PSANP fraction of *P. polycephalum* is similar to that in acidic nuclear proteins of rat liver [1.0 percent in *P. polycephalum* compared to 0.94 percent in rat liver (*8*)]. The alkali-labile phosphate in PSANP increases from 0.85 percent after the 3-hour S period to 1.0 percent at early prophase. Incorporation studies with [^{32}P]orthophosphate show a 50 percent increase in radioactivity in the PSANP during the G_2 period, results reflecting the net increase in phosphate and perhaps an active turnover of existing nonlabeled phosphate.

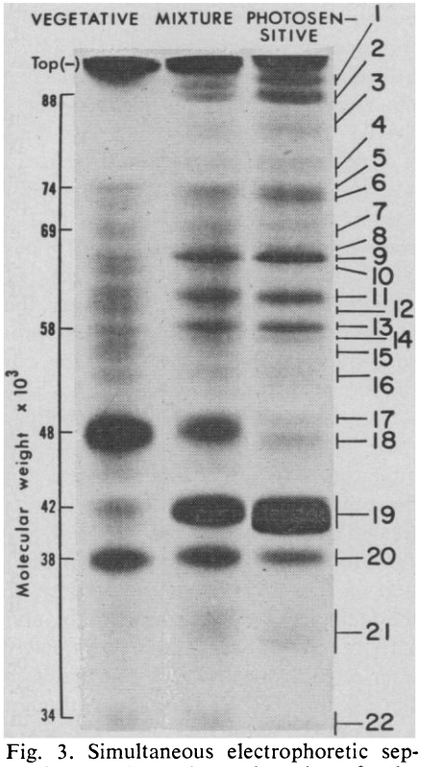

Fig. 3. Simultaneous electrophoretic separation and coelectrophoresis of the PSANP present in the vegetative plasmodium and after development of the photosensitive state in *P. polycephalum*. For each profile, 160 μg of protein complexed with SDS was subjected to electrophoresis for 20 hours at 3 ma per gel through 6-mm, 10 percent polyacrylamide gels containing 0.1 percent SDS in 0.1*M* phosphate buffer, *p*H 7.4 (which was identical to the reservoir buffer). The proteins were fixed and stained with 30 percent acetic acid containing 1 percent Amido black 10B. The distance from the top of the gels to band 22 is 4.7 cm. Under these conditions the tracking dye and several fast-moving proteins have eluted into the lower buffer solution. Molecular weights were determined from the linear relationship between R_F and the logarithm of the molecular weight (*24*) of the following protein standards: bovine hemoglobin monomer, lysozyme, myoglobin, creatine kinase monomer, and deoxyribonuclease.

The PSANP fraction is rapidly synthesized after mitosis (Fig. 1), and no differences can be detected in the electrophoretic profiles of the PSANP isolated at 18 time points during the 8-hour mitotic cycle. However, the development of the photosensitive state during differentiation (Fig. 3) is marked by the appearance of protein bands 1, 2, 9, 11, 13, and 19 and the almost complete disappearance of a major protein banding in the region of bands 17 and 18. This new electrophoretic profile is unchanged when sporangial stalks are formed 8 hours after illumination. If the photosensitized plasmodia are again fed, the electrophoretic profile characteristic of vegetative growth returns within 24 hours, and the plasmodium can no longer be induced by light to sporulate. It has not been determined whether bands 11 and 13 are new proteins synthesized during development of the photosensitive state or whether, as judged by densitometer scans, they are simply three- to fourfold increases in existing proteins. The latter does appear to be the case with bands 3, 4, and 5. In all sporulation studies, more than 98 percent of the control cultures sporulated after the 4-hour illumination period.

Changes in the electrophoretic profile of the PSANP fraction are associated with a new cell state in the plasmodium and must occur before differentiation. While these changes may be interpreted as the result rather than the cause of alterations in gene expression, published reports suggest the latter interpretation. The demonstration that estrogen stimulates the synthesis of specific acidic nuclear proteins in rat uterus (*9*), hydrocortisone stimulates the synthesis of a specific acidic nuclear protein in rat liver (*7*), and increased RNA synthesis is associated with the newly synthesized proteins indicates that these acidic nuclear proteins may have a direct role in gene expression.

In *P. polycephalum* the photosensitive state is induced by transferring the actively growing plasmodium to a starvation (*21*) medium containing niacin and salts (*20*) and incubating for at least 4 days in darkness. The energy necessary for coalescence, stalk formation, meiosis, pigment and spore wall formation, and other activities associated with differentiation must therefore be derived from reoriented metabolism within the plasmodium. This dramatic reorientation of energy metabolism, together with the synthesis of new proteins involved with sporulation (*22*), require the presence of perhaps numerous control mechanisms. The demonstration, then, that within a specific class of nuclear proteins (unchanging throughout somatic growth) new proteins appear and existing proteins disappear during differentiation suggests that the PSANP may be involved in a control mechanism involving gene expression. The dependence of sporulation on synthesis of protein (*22*) and RNA (*23*) points to the existence of transcriptional control mechanisms operating in *P. polycephalum* during sporulation.

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12. Abbreviations are as follows: G_1, the period of interphase before DNA synthesis; G_2, the period after DNA synthesis; S, the period when DNA is synthesized.
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19. For the G_2 period, the percentage of the total dry weight of nuclear material constituted by the PSANP fraction (6.5 percent) was determined in triplicate. Hemocytometer counts were performed on two equal por-

tions of purified nuclear suspensions. The nuclei from one portion were dried to constant weight under vacuum over P_2O_5 and the total PSANP fraction was extracted from the second portion. Protein was determined by the method of Lowry *et al.* (*20*). For the G_2 period, the percentage of dry weight of nuclear material constituted by DNA (5.5 percent) was determined as above. Before DNA analysis the nuclear RNA was removed from the nuclear pellets with 0.4*N* NaOH, and the DNA was extracted as previously described [W. Sachsenmaier, H. P. Rusch, *Exp. Cell Res.* **36**, 124 (1964)]. The DNA content was determined by its optical density at 260 nm and by the diphenylamine test [K. Burton, *Biochem. J.* **62**, 315 (1956)].

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Snake Neurotoxins: Effect of Disulfide Reduction on Interaction with Electroplax

Abstract. *Reduction of disulfide bonds of cobra neurotoxin by dithiothreitol results in decreased activity on the electroplax preparation. Activity is restored completely after reoxidation by 3,3′-dithiobis[6-nitrobenzoic acid]. Reduction of the disulfide bonds in the vicinity of the receptor does not decrease the effect of cobrotoxin.*

A great deal of effort has been concentrated on purifying and elucidating the structure and toxicology of snake neurotoxins. The amino acid sequence (*1*) and the number and position of disulfide bonds (*2*) of several toxins have been determined, and some preliminary x-ray crystallography studies of erabutoxin have been reported (*3*). It was shown that the basic structure of erabutoxins, cobrotoxin, and bungarotoxins is the same and that only some amino acid positions are variable (*1*).

Changeux *et al.* showed that α-bungarotoxin acts somewhat like curare at the synapses of the electroplax, the only differences being its greater potency and its irreversibility (*4*). Miledi *et al.* (*5*) used bungarotoxin and Meunier *et al.* cobrotoxin (*6*) to isolate a protein fraction which showed AcCh-receptor binding characteristics. Earlier studies have shown that the tryptophan residue in position-29 (*7*) and the integrity of the disulfide bonds (*8*) are essential for activity. These experiments were done on whole animals and nothing was known about the tertiary structure of the toxins and the nature of receptors. Therefore, these experiments were repeated on the electroplax preparation (*9*) where much information about the receptor has been obtained during the last decade.

X-ray crystallography suggests that the erabutoxin may be a flattened, disk-shaped molecule with little helical structure (*3*). The tryptophan residue in position-29 and the disulfide linkage 3 to 24 are very likely at the surface of the molecule. The distance between the tryptophan residue and this disulfide bridge could be as little as 10 Å. Hence the tryptophan residue might possibly act as the affinity binding site and a disulfide exchange between the toxin and the disulfide bond of the receptor site may take place. Reduction of the receptor with dithiothreitol (DTT) has been shown to reduce the effects of carbamylcholine (*10*). However, the reduction of the receptor did not prevent the irreversible action of the cobrotoxin, and neither 3,3′-dithiobis[6-nitrobenzoic acid] (DTNB) nor cholinethiol could cause a reversal of the inhibitory effect of the neurotoxin. On the other hand, if the neurotoxin was treated with $5 \times 10^{-4}M$ DTT at *p*H 8.0 for 10 minutes before the application to the electroplax, the activity of the toxin was reduced considerably, and activity could be restored completely after reoxidizing the stock solution with $1 \times 10^{-3}M$ DTNB (Fig. 1). Although a disulfide interaction between toxin and receptor has not been ruled out completely by these experiments, it seems more likely that a complemen-

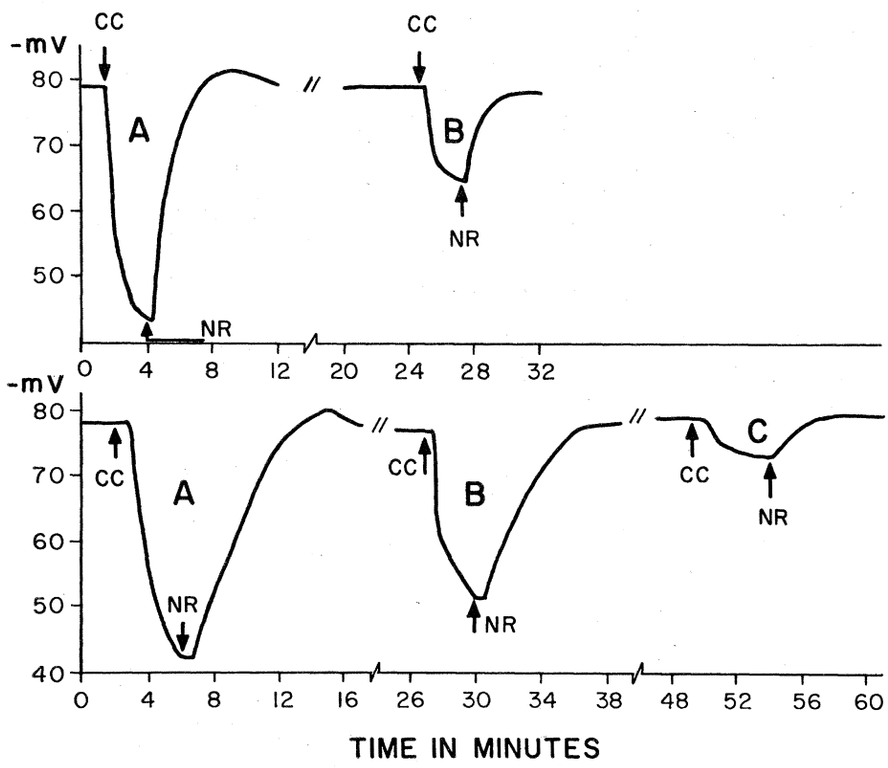

Fig. 1. Potential recording of the innervated membrane of a single electroplax from *Electrophorus electricus*. (Top A) Control response to $5 \times 10^{-5}M$ carbamylcholine (*CC*). (Top B) Reduced response to $5 \times 10^{-5}M$ carbamylcholine after the cell was exposed to cobrotoxin (0.001) mg/ml) for 10 minutes. (Bottom A) Control response to $5 \times 10^{-5}M$ carbamylcholine. (Bottom B) Response to carbamylcholine $5 \times 10^{-5}M$ after the cell was exposed to cobrotoxin (0.001 mg/ml) for 10 minutes which was incubated previously with $5 \times 10^{-4}M$ DDT as stock solution (0.1 mg/ml) for 10 minutes, *p*H 8.0. (Bottom C) Same as B, except that the cell was exposed to the same toxin which was reoxidized with $1 \times 10^{-3}M$ DTNB at *p*H 7.8. *NR*, normal Ringer solution.