PHYSARUM WORKSHOP 1976

The next *Physarum* meeting will take place from September 14 to 16, 1976, in the vicinity of Berne. It will most likely be held at a simple, but charming old hotel at Rütihubelbad some 20 kilometers from Berne, amidst the rolling hills of the Emmenthal.

As the meeting will start at 9 a.m. on Tuesday morning, it would be wise to plan to arrive on Monday evening. Depending on the program, we would hope to make an outing on Wednesday afternoon.

The hotel will cost approx. 35.- francs per day. If you need further registration forms, please make photocopies. It may be possible for us to obtain a modest grant for travel allowances.

For registration and further information, contact:

Prof. R. Braun
Institute for General Microbiology
of the University of Berne
Altenbergrain 21
CH-3013 Berne, SWITZERLAND
(Phone: 031 / 42 20 58)

REPORT ON THE GAINESVILLE MEETING

The sixth *Physarum* conference was held at the University of Florida, November 8-10, 1975. Thirty scientists from the U.S., Canada and Germany participated.

The meeting began with a myxomycete hunting expedition on Saturday, followed by a sea food dinner at Cedar Key on the Gulf coast. After ten half-hour paper presentations on Sunday (molecular biology and genetics), the participants adjourned to the Aldrich home for refreshments before the traditional conference dinner. Ten reports were presented on Monday (six on ultrastructure, four on cell cycle and differentiation). For those who did not attend, abstracts are included with this mailing of the newsletter.

Most participants stayed at the J. Wayne Reitz Union, an outstanding facility located on the campus of the University of Florida. They enjoyed fine hospitality during their stay in Gainesville. The hard work of Henry Aldrich and his associates Mike Dykstra and Bill Dougherty is greatly appreciated. It was an excellant conference.

MAILING FUND REPORT

Balance, 12/31/74		$192.82
Receipts:		
Contributions	172.40	
Interest	11.40	
	183.80	183.80
		376.62
Disbursements:		
Mailing costs	103.07	103.07
Balance, 12/31/75		$273.55

TITLES AND SUMMARIES IN PRINT

SCIENCE, VOL. 190, 65, OCTOBER 1975

Life Cycle Variants of *Physarum polycephalum* That Lack the Amoeba Stage

Abstract. *The myxomycete life cycle ordinarily proceeds in the sequence plasmodium-spore-amoeba-plasmodium. Extraordinary variants are described in which the sequence is plasmodium-spore-plasmodium.*

PAUL N. ADLER
LANCE S. DAVIDOW
CHARLES E. HOLT
Department of Biology, Massachusetts Institute of Technology, Cambridge 02139

Eur. J. Biochem. 57, 361 – 369 (1975)

Circular DNA and Rolling Circles in Nucleolar rDNA from Mitotic Nuclei of *Physarum polycephalum*

Hans-Jürgen BOHNERT and Barbara SCHILLER
Botanisches Institut der Universität Düsseldorf

Roswita BÖHME and Helmut W. SAUER
Fachbereich Biologie der Universität Konstanz

1. About 15% of nucleolar DNA (1.712 g/cm^3) from *Physarum polycephalum* displaying maximum hybridization to ribosomal RNA, is composed of circular DNA of 3.9 ± 0.2 μm contour length or multiples thereof.

2. A portion of these circular molecules (25%) contained linear DNA pieces longer than circumference length. In a small fraction of circular DNA linear pieces, shorter than the unit length, were observed.

3. Most nucleolar DNA, [^{3}H]thymidine-labeled or hybridizable to ribosomal RNA was separable from chromosomal DNA during G_2 phase, mitosis and S phase of the cell cycle.

4. Ribosomal DNA content was not amplified during the cell cycle, was unchanged during exponential or stationary growth phase and amounted to about 0.11 – 0.21% of nuclear DNA in diploid and hexaploid strains of *Physarum* or 100 – 200 ribosomal genes per diploid genome.

FLUCTUATIONS IN DEOXYRIBO- AND RIBONUCLEOSIDE TRIPHOSPHATE POOLS DURING THE MITOTIC CYCLE OF *PHYSARUM POLYCEPHALUM*

KIRSTEN FINK*

Enzyme Division, University Institute of Biological Chemistry B, Copenhagen (Denmark)

Biochimica et Biophysica Acta, 414 (1975) 85–89

Summary

Fluctuations in the pools of deoxyribo- and ribonucleoside triphosphates have been measured during the synchronous mitotic cycle of the slime mould *Physarum polycephalum*.

The most pronounced fluctuation of the deoxyribonucleoside triphosphates was seen shortly before and after initiation of DNA synthesis. The pools of dTTP, dATP, dCTP and dGTP expanded before initiation of DNA synthesis and decreased again in early S phase.

The pools of ribonucleoside triphosphates increased during mitosis and again 1 h after mitosis and 5 h after mitosis.

SOME EVIDENCE FOR REPLICATION-TRANSCRIPTION COUPLING IN *PHYSARUM POLYCEPHALUM*

H. FOUQUET, R. BÖHME, R. WICK, H. W. SAUER*
Universität Konstanz, Fachbereich Biologie,
D-775 Konstanz, GFR

AND K. SCHELLER
Institut für Physiologische Chemie,
Universität Marburg, Deutschhausstr. 1–2,
D-355 Marburg, GFR

J. Cell Sci. **18**, 27–39 (1975)

SUMMARY

Hydroxyurea, at concentrations of 40–60 mM, selectively and effectively blocked incorporation of thymidine into DNA. Inhibition occurred within 5–10 min of application of the agent when DNA synthesis was in progress, while the onset of replication at the beginning of *S*-phase and DNA synthesis in G_2 phase were not affected.

Uridine incorporation into TCA-precipitable material, in the presence of hydroxyurea, was significantly (up to 70 %) inhibited in early *S*-phase of the cell cycle. Selective inhibition of RNA synthesis was confirmed for RNA separated into rRNA-rich and poly-(A)-rich RNA fractions and analysed by the 2 kinds of DNA–RNA hybridization reactions. Uridine incorporation into poly (A) RNA was also inhibited under conditions where cycloheximide prevented maturation of nascent DNA molecules in early *S*-phase.

We assume that chromatin which is replicating early DNA sequences may be a more competent template for transcription.

Nature, Vol. 255, No. 5505, pp. 253–255, May 15, 1975

Variable redundancy in RNA transcripts isolated in S and G_2 phase of the cell cycle of *Physarum*

H. FOUQUET
H. W. SAUER

*Universität Konstanz,
Fachbereich Biologie,
GFR 775 Konstanz,
Postfach 7733, West Germany*

ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS 168, 273–280 (1975)

Effects of Cordycepin on RNA Synthesis in *Physarum polycephalum*

H. FOUQUET, R. WICK, R. BÖHME, AND H. W. SAUER

Universität Konstanz, Fachbereich Biologie, D-775 Konstanz, GFR

AND

K. SCHELLER

Institut Für Physiologische Chemie Universität Marburg, Deutschhausstraße 1-2 D-355 Marburg, GFR

Cordycepin (100–200 μg/ml) blocked synthesis of all species of RNA separable by gel electrophoresis and by cellulose chromatography, similarly to actinomycin D, but more efficiently and rapidly. At low concentrations (40–80 μg/ml) cordycepin inhibited predominantly ribosomal RNA synthesis in *Physarum*, like toyocamycin, another adenosine analog. In nuclear preparations polyadenylylation of RNA was not affected by cordycepin. However, in the presence of cordycepin, no poly(A) RNA was found in the polysome fraction.

Volume 2 number 8 August 1975 — Nucleic Acids Research

DNA replication in Physarum polycephalum: characterization of replication products in vivo

Steinar Funderud and Finn Haugli

Institute of Medical Biology, University of Tromsø, Tromsø, Norway

ABSTRACT

Synchronous plasmodia of Physarum polycephalum in DNA synthesis were pulse-labelled with [^{3}H]- thymidine for time periods of 15 seconds up to 9 minutes, or given a 30 seconds pulse followed by chase periods of 9 minutes up to 6 hours. Sedimentation analysis in alkaline sucrose gradients revealed at least five species of single stranded DNA molecules in the pulse experiments. Co-sedimentation of [^{14}C]-labelled phage-DNA gave relative S-values of 5-7, 13-15, 23-25, 30 and 33-35 for these DNA molecules, all of which can be chased into DNA of higher molecular weight.

The function of slime from *Physarum flavicomum* in the control of cell division[1]

HENRY R. HENNEY, JR., AND MORTAZA ASGARI
Department of Biology, University of Houston, Houston, Texas 77004

HENNEY, H. R., JR., and M. ASGARI. 1975. The function of slime from *Physarum flavicomum* in the control of cell division. Can. J. Microbiol. 21: 1866–1876.

A haploid cell of the myxomycete *Physarum flavicomum* undergoes cytokinesis, producing a large population of cells. However, after syngamy, cytokinesis no longer occurs but karyokinesis does and subsequent growth results in the formation of a diploid syncytial plasmodium. Slime, which is produced by the plasmodium but not the haploid cells, was aseptically isolated and purified, and tested for its effect as a cytokinetic regulator. Slime (a viscous, high molecular weight, acidic glycoprotein) affected cytokinesis of the haploid myxamoebae growing in pure culture in soluble media, and the effect was concentration dependent. In simple media, a slime concentration of about 6×10^{-5} μg protein per cell suppressed cytokinesis about 50%, unequally inhibited the synthesis of protein, RNA, and DNA, but stimulated respiration. The biological activity of slime was not species specific and it also affected the bacterium *Bacillus subtilis* by inhibiting cytokinesis, stimulating oxygen uptake, and producing an aberrant cell morphology. Slime was inactivated by heat, fragmentation, and incubation with dithiothreitol, mercaptoethanol, and the proteolytic enzyme papain (EC 3.4.22.2). The inhibitory effect of slime on cell division of haploid cells could not be achieved using mucin or various polyanions. The possible role of slime in the production of the diploid syncytium is discussed.

Nutritional Control of Differentiation (Sclerotization) of the Myxomycete *Physarum flavicomum*

HENRY R. HENNEY, JR. AND GLENNA MAXEY
Department of Biology, University of Houston, Houston, Texas 77004

Henney, H. R., Jr. & Maxey, G. (1975) Nutritional Control of Differentiation (Sclerotization) of the Myxomycete *Physarum flavicomum*. *Can. J. Biochem.* 53, 810–818

During differentiation (sclerotization) of the Myxomycete *Physarum flavicomum*, the acellular plasmodium converts into numerous dormant cells surrounded by cell walls. This work establishes that a condition of nutrient imbalance triggers the differentiation process. Specifically, the unavailability of an adequate spectrum of amino acids in the medium initiates the metabolic and morphological alterations characteristic of the sclerotizing plasmodium.

In the absence of extracellular amino acids, the cellular pool of amino acids and cellular protein were catabolized as differentiation proceeded. The pattern of amino acids in the cellular pool also changed during differentiation, as the content of pool amino acids was reduced at least 75%. The decrease in protein content was negligible after 12 h incubation but was about 40% at 48 h when differentiation was complete. However, in the presence of extracellular amino acids, protein degradation, amino acid pool depletion, and differentiation were all inhibited. Ammonium ions (12.4 m*M*) similarly delayed differentiation.

Differentiation, amino acid pool depletion, and the degradation of cellular protein readily occurred in the presence of an extracellular supply of dextrose, which stimulated cell wall formation. The effect of dimethyl sulfoxide, cyclic 3′,5′-adenosine monophosphate, glutathione, diamide, and other compounds on the differentiation process are reported also.

Amino Acid and Protein Metabolism During Differentiation (Sclerotization) of the Myxomycete *Physarum flavicomum*

HENRY R. HENNEY, JR., AND GLENNA MAXEY
Department of Biology, University of Houston, Houston, Texas 77004

Henney, H. R., Jr. & Maxey, G. (1975) Amino Acid and Protein Metabolism During Differentiation (Sclerotization) of the Myxomycete *Physarum flavicomum*. *Can. J. Biochem.* 53, 834–843

Protein synthesized by growing plasmodia of *Physarum flavicomum* was steadily degraded when the plasmodia were induced to differentiate (form sclerotia). Protein synthesis occurred during the initial one-fifth (9 h) of the 48 h differentiation period, but most of this protein was also degraded shortly after its synthesis. Amino acids were primary catabolites during the differentiation process, and catabolism was extensive, even in the presence of dextrose. Glutamic acid was catabolized at a rate about two and a half or three times greater, respectively, than that observed for valine and arginine. Active transport systems for amino acids appeared to be present and to remain functional in *P. flavicomum* during differentiation. Amino acids included in the sclerotization media were rapidly accumulated into the cell pool and protein fractions. Intracellular amino acids were actively retained and were not released into the medium during differentiation.

Differentiation of this Myxomycete, therefore, is characterized by a change in the metabolism of the sclerotizing plasmodium to an autolytic type, as cellular proteins and amino acids are actively catabolized during the formation of the dormant sclerotia.

Aminopeptidases of *Physarum polycephalum*

ACTIVITY, ISOENZYME PATTERN, AND SYNTHESIS DURING DIFFERENTIATION*

WOLF HOFFMANN‡ AND ALOYS HÜTTERMANN
From the Forstbotanisches Institut der Universität Göttingen, West Germany

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 250, No. 18, Issue of September 25, pp. 7420–7427, 1975

In extracts from both growing and differentiating (spherulating) plasmodia of the true slime mold *Physarum polycephalum*, high aminopeptidase activities were found. The specificity of the aminopeptidases changed during differentiation with a higher relative activity towards hydrophobic NH_2-terminal amino acids. This change in specificity was found to be the result of a shift in the isoenzyme spectrum during differentiation as was tested by isoelectric focusing in sucrose gradients. Three different classes of isoenzymes were found: one band which was present in both growing and differentiating cultures; two bands which were found only in growing cultures; and four bands which were detectable only in differentiating plasmodia. If cycloheximide was applied during the induction of differentiation, only one band, the one present in both types of plasmodia, was found in the isoelectric focusing.

Density labeling experiments using deuterated amino acids revealed that the bands which are present in differentiated plasmodia only are synthesized *de novo* during this differentiation.

ACTIVITY, ISOENZYME PATTERN, AND SYNTHESIS OF UDPGLUCOSE 4-EPIMERASE DURING DIFFERTIATION OF *PHYSARIUM POLYCEPHALUM*

ALOYS HÜTTERMANN, MARLIES GEBAUER, IRMGARD WESSEL and WOLF HOFMANN

Forstbotanisches Institut der Universität Göttingen, Göttingen (G.F.R.)

Biochimica et Biophysica Acta, 384 (1975) 493—500

Summary

1. The specific activity of UDPglucose 4-epimerase (EC 5.1.3.2) increases by about 50% during the first 24 h of starvation-induced differentiation (spherulation) of *Physarum polycephalum*.

2. At all stages during differentiation, the enzyme activity is very sensitive to actinomycin-C and cycloheximide, inhibitors of transcription and translation, with a half life against cycloheximide of about 20 min (if added 12 h after the induction of differentiation).

3. The isoenzyme pattern, as revealed by isoelectric focusing in sucrose gradients, does not change during spherulation. One main band with a p*I* of 6.7, with a shoulder (p*I* 7.6) and a minor band (p*I* 6.0) was observed in extracts both from growing and differentiating cultures.

4. Density labelling experiments using deuterated amino acids with subsequent analysis by equilibrium density gradient sedimentation in 15—35% (w/w) metrizamide gradients revealed a rather slow rate of enzyme synthesis, which is in contrast to the observed high sensitivity against actinomycin-C and cycloheximide.

Naturwissenschaften 62, 283–289 (1975)

Neuere Forschungen über Zellzyklus und Kernteilung

am Schleimpilz *Physarum polycephalum*

Brigitte M. Jockusch*

Max-Planck-Institut für Biologie, Abt. Melchers, Tübingen

The plasmodial stage of *Physarum polycephalum* contains up to 10^9 nuclei which undergo a naturally synchronous mitosis every 8 h. Nuclear processes such as DNA and RNA synthesis as well as many cytoplasmic processes such as histone synthesis are also synchronous. *Physarum polycephalum* is therefore widely used in studies of cell-cycle events. This article describes experiments that may help to explain two fundamental biological processes: (1) the mechanism that triggers mitosis. (2) the structural basis of mitotic movement.

ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS 170, 49–60 (1975)

Nuclear Phosphoproteins of *Physarum polycephalum*

Characterization and Phosphorus Content of the Phenol-Soluble Nuclear Acidic Proteins[1]

BRUCE E. MAGUN, RICHARD R. BURGESS, AND HAROLD P. RUSCH

McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin and Department of Anatomy, University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38163

AND

McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin

The phenol-soluble nuclear phosphoproteins of the slime mold *Physarum polycephalum* have been characterized using ^{32}P isotopic labeling followed by electrophoresis on sodium dodecyl sulfate–polyacrylamide gels. Plasmodia were labeled continuously with ^{3}H-amino acids and $^{32}P_i$ before extraction of the phenol-soluble nuclear phosphoproteins, which were then separated by acrylamide gel electrophoresis. After scintillation counting of gel slices, we were able to calculate the *moles phosphate per mole polypeptide* in individual gel slices.

The results indicated minimum values of less than 2 phosphates per polypeptide for most resolvable ^{32}P bands. The phenol-soluble nuclear acidic protein fraction contained less than 0.1% phosphorus by weight. Gel autoradiographs demonstrated that most of the phosphoprotein peaks did not correspond to major protein peaks, a situation that was also found in autoradiographic profiles of other nuclear protein fractions. Pronase digestion of samples before electrophoresis abolished the appearance on gels of bands with ^{32}P activity.

The results of this investigation suggest that in *Physarum* the phenol-soluble nuclear acidic proteins appear to be similar to other nuclear proteins in their phosphoprotein composition. The evidence presented suggests that phosphoproteins comprise a small part of the complement of nuclear acidic proteins.

Experimental Cell Research 95 (1975) 405–415

THE CONTROL OF MITOSIS IN *PHYSARUM POLYCEPHALUM*

The Effect of Lowering the DNA : Mass Ratio by UV Irradiation

P. E. SUDBERY[1] and W. D. GRANT

Department of Genetics, University of Leicester, Leicester, LE1 7RH, UK

SUMMARY

A model for the control of mitosis is presented and, along with four other models described previously, is tested by the response of *Physarum polycephalum* to UV irradiation. Plasmodia were irradiated following the second mitosis (M II) after fusion of microplasmodia. As shown by other authors, the onset of the next mitosis (M III) was delayed but the period M III–M IV was shortened relative to control plasmodia. It is shown that the period M III–M IV cannot be shortened beyond a minimum of 6 h despite increasing doses of UV. This minimum length is shown to be relatively independent of growth rate. If conditions were such that the length of M III–M IV was shortened to this minimum value the length of MIV–MV was also shorter than the corresponding control period. If the period M II–M IV was longer than the minimum following irradiation then the length of M IV–M V was not shortened. It is argued that only the latter situation allows models to be tested and it is shown how the observed result is consistent with only two of the five models considered. A further test compared the length of M III–M IV under these conditions with that predicted from the amount of DNA destroyed by the UV. This result was consistent only with the same two models.

ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS 172, 224–229 (1976)

Poly(Adenosine Diphosphate Ribose) Glycohydrolase in *Physarum polycephalum*[1]

MIYOKO TANAKA, MASANAO MIWA, TAIJIRO MATSUSHIMA, TAKASHI SUGIMURA, AND SYDNEY SHALL

Biochemistry Division, National Cancer Center Research Institute, Chuo-ku, Tokyo 104, Japan, Department of Molecular Oncology, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108, Japan, and Biochemistry Laboratory, University of Sussex, Brighton, England

Poly(adenosine diphosphate ribose) glycohydrolase, which has thus far only been found in mammalian tissues, was found for the first time in the primitive eukaryotic slime mold *Physarum polycephalum*. The hydrolytic product of poly(adenosine diphosphate ribose) with this enzyme was identified as adenosine diphosphate ribose by paper and thin-layer chromatography. It is likely that the enzyme caused exoglycosidic hydrolysis. The optimal pH of this enzyme was 6.0, and the K_m value was 4.3 μM, as adenosine diphosphate ribose residues of polymer. Adenosine diphosphate ribose, ADP and ATP at a concentration of 0.1 mM strongly inhibited the enzyme activity. 3′,5′-Cyclic AMP was inhibitory at a concentration of 1 mM. The molecular weight of this enzyme was estimated to be 57,000.

Amoebal culture is fast becoming an exact science. According to A. Hüttermann, the aqueous component of axenic medium should contain 99 parts triply-quartz-distilled water and 1 part Göttingen tap water . . .

PREMATURE REPLICATION OF LATE S PERIOD DNA REGIONS IN EARLY S NUCLEI TRANSFERRED TO LATE S CYTOPLASM BY FUSION IN *PHYSARUM POLYCEPHALUM*

JOHN J. WILLE, Jr* and STUART A. KAUFFMAN**

Department of Biophysics and Theoretical Biology, The University of Chicago, Chicago, Ill. 60637 (U.S.A.)

Biochimica et Biophysica Acta, 407 (1975) 158—173

Summary

Fusion of a late S period plasmodium of *Physarum polycephalum* to an early S period plasmodium causes premature replication of late S replicating regions in the nuclei of the early S plasmodium. The extent of ahead-of-schedule replication of late S replicating regions in early S period nuclei increases to a plateau of 16—20% for fusions with 40—70 min of phase difference, then declines for larger phase differences. The stimulatory factors for late S replicative units are present only in late S plasmodia and appear to act only on late S regions. Once replicated, early S replicating regions are not stimulated to replicate again by fusion to a plasmodium entering the S period. Our data do not discriminate between anti-termination of replication by factors of stop sites on long replicons, and a sequential initiation of replication on new, possibly non-adjacent regions, but does provide evidence that the stimulatory factors are distinct from one another and specific for certain target replicative units.

Nature Vol. 256, 413–414, *July 31 1975*

Sporulation-inducing factor in slime mould *Physarum polycephalum*

William M. Wormington
Chong G. Cho
Robert F. Weaver

Department of Biochemistry, McCollum Laboratories, University of Kansas, Lawrence, Kansas 66044

Nature, Vol. 257, No. 5525, pp. 422–423, October 2, 1975

DNA breakage caused by dimethyl mercury and its repair in a slime mould, *Physarum polycephalum*

Randall W. Yatscoff
Joseph E. Cummins

Department of Plant Sciences, The University of Western Ontario, London, Ontario, Canada

TITLES AND SUMMARIES IN PRESS

ADVANCE OF MITOSIS BY HISTONE PHOSPHOKINASE

E.M. Bradbury, D.G. Hardie, R.J. Inglis, H.R. Matthews

Department of Physics, Portsmouth Polytechnic,
Gun House, Hampshire Terrace, Portsmouth, UK

and T.A. Langan

Department of Pharmacology, University of Colorado
School of Medicine, 4200 East 9th Avenue
Denver, Colorado 80220, USA

SUMMARY

The previous observation that growth associated histone kinase (HKG) from Ehrlich ascites cells brings forward mitosis in *Physarum polycephalum* has been confirmed with more step 1 histone kinase and a more purified (step 2) histone kinase and the statistical significance of the results assessed. The mitosis appears normal in the phase contrast microscope and DNA synthesis is initiated after mitosis as usual. *In vitro* the growth associated histone kinase phosphorylates chromatin, the phosphate appearing in F1 histone. The results are interpreted as providing support for the hypothesis that growth associated histone kinase controls the initiation of mitosis through F1 histone phosphorylation and chromosome condensation.

Experimental Cell Research, in press

FACTORS AFFECTING THE MOVEMENT OF SLIME MOLD PLASMODIA

John R. Denbo and Donald M. Miller

Department of Physiology, Southern Illinois University
Carbondale, Illinois 62901

ABSTRACT. Migration rate of oriented plasmodia of *Physarum polycephalum* is determined by environmental parameters of substratum osmolarity, pH, temperature, and specific ion concentrations. 2. Migration occurred between substratum osmolarities of 0-340 mOsmoles, pH values of 2.35-12.0, and temperatures of $8^{o}C$-$37^{o}C$ (optimum for our experimental conditions, $24^{o}C$). 3. Except for rubidium, common metallic cations depressed migration rate over and above their concentration effect, as follows in decreasing order of effectiveness: Mg^{++} $Ca^{++}Li^{+}$, Na^{+}, K^{+}, Rb^{+}. 4. Data obtained in these experiments are interpreted to demonstrate that the sol-gel equilibrium of the plasmodium is the major determinant of migration rate.

Journal of Comparative Biochemistry and Physiology, in press

Naturwissenschaften, in press

Differential Expression of RNAse Activities in the Life Cycle of *Physarum polycephalum*

Gesche Brand and A. Hüttermann

Forstbotanisches Institut der Universität Göttingen

F. B. Haugli

Institut for Medisinsk Biologi, Universitet i Tromsø

Most eukaryotic organisms have a complex life cycle in which haploid and diploid stages usually show a different morphology. An organism which is well suited for a study of the biochemical basis for these differences, which probably occur in identical genetical background, is the true myxomycete *Physarum polycephalum* (review in [1]).

We used the strain Colonia, which is either homothallic [2] or apogamic [3]; the amoebae from this strain can form plasmodia with an identical genome (either haploid or diploid). Thus, complications in the interpretation of biochemical data due to segregation of chromosomes and new genetic combinations are avoided (for a detailed review of *Physarum* genetics *cf.* [4]).

When extracts were made from growing plasmodia of this strain and analyzed by isoelectric focusing in sucrose gradients [5], three isoenzyme bands were found in the acidic region of the pH gradient with isoelectric points of 3.9, 3.7, and 3.4 (mean values of ten different analyses; in individual experiments, we found a variation of about ±0.2 pH for the different bands, the pattern always being the same) (Fig. 1a). This indicates that in growing plasmodia of this strain at least three different RNAse isoenzymes are present which are all acidic. When extracts made from growing amoebae of the same strain, grown on formaldehyde-inactivated *Escherichia coli* [6], were analyzed by the same method, a different RNAse pattern was observed: only main band was observed in the acidic region with a pI of 3.4, and a second band, in the neutral region, with a pI of 6.2 (Fig. 1b). From this it is evident that two of the three acidic RNAses present in the plasmodial stage are very much reduced in their relative activity in the growing amoebae. On the other hand, one neutral RNAse band was found in the amoebae which is absent in the plasmodia. We could exclude the possibility that this band is an artefact due to a contamination of the extract with the food bacteria. This was tested by measuring the RNAse activity present in the formaldehyde-inactivated *E. coli*. In an extract made from 10^{10} cells, no RNAse activity was detectable under our assay conditions.

The different RNAse isozyme patterns as shown in Fig. 1 were obtained from amoebae and plasmodia which have an identical genome. The changes observed must therefore be associated with *different gene expression in the different stages of the life cycle of Physarum polycephalum*. The presence of such differences has been suggested from studies on the acidic nuclear proteins from amoebae and plasmodia of the heterothallic "Wisconsin"-strain of *Physarum polycephalum* [7]. We have studied the RNAse isozymes during the life cycle of this strain too and found the same RNAse pattern in plasmodia as shown in Fig. 1a and in amoebae as shown in Fig. 1b. Our findings agree with the hypothesis that different sets of genes may be active in the amoebae and plasmodia of *Physarum polycephalum* [1], which is based on the observation that some temperature-sensitive mutations isolated as amoebae from the homothallic strain are not expressed in the plasmodia.

This work was supported by grants from the Deutsche Forschungsgemeinschaft and the Universitet i Tromsø.

Received May 15 and July 18, 1975

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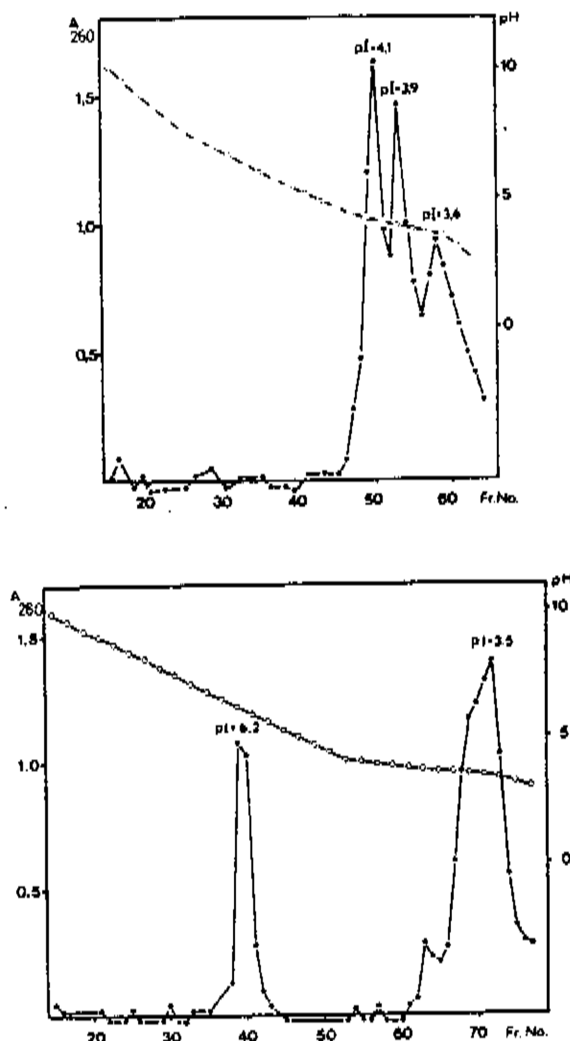

Fig. 1. (a) Enzyme pattern of RNAses in extracts from plasmodia of *Physarum polycephalum*, strain, Colonia. The plasmodia were grown, harvested, and homogenized as described [8]. 5 ml enzyme extract, containing about 30 mg protein, were loaded on isoelectric focusing column, using the published method [5]. After the run, fractions were analyzed for the pH and the RNAse activity [9]. The upper curve indicates the pH gradient (right ordinate), the left ordinate indicates the activity scale expressed increase in absorbance unit in the assay. (b) Isoenzyme pattern of RNAses in extracts from amoebae of the homothallic strain Colonia, isogenic with the plasmodia analyzed in (a). 5×10^8 amoebae were grown of formaldehyde-inactivated *E. coli*, Homogenized with ultrasonication and analyzed as described for (a)

EFFECTS OF CYCLOHEXIMIDE ON THYMIDINE METABOLISM AND ON DNA STRAND ELONGATION IN *PHYSARUM POLYCEPHALUM*

Helen H. Evans, Sandra R. Littman,
Thomas E. Evans, and Eugene N. Brewer

Departments of Radiology, Biochemistry and
Microbiology, Case Western Reserve University
Cleveland, Ohio 44106

SUMMARY

Treatment of *Physarum polycephalum* with cycloheximide during the S period resulted in a reduction in the incorporation of [^{3}H]thymidine into DNA. This effect was caused by both a reduction in the specific activity of TTP and by an inhibition of progeny strand elongation within replication units. No effect of the drug on the initiation of synthesis of replication units or on the ligation of DNA fragments was detected.

Journal of Molecular Biology, in press

EFFECTS OF EXTREMELY LOW FREQUENCY ELECTROMAGNETIC FIELDS ON *PHYSARUM polycephalum*

E.M. Goodman, Ben Greenebaum and Michael T. Marron

Division of Science
University of Wisconsin-Parkside Campus
Kenosha, Wisconsin 53140

ABSTRACT. Microplasmodia from the slime mold *Physarum polycephalum* have been continuously exposed to weak electromagnetic fields at 45, 60 and 75 Hz. To date, microplasmodia have been exposed to fields of 75 Hz, 2.0 G, 0.7 V/m for more than 700 days. Two other sets of cultures have been exposed to 45 and 60 Hz fields (2.0 G, 0.7 V/m) for 180 and 400 days, respectively. The time between successive mitotic divisions in cultures exposed to fields varied from 0.5 to 2 hours longer than their respective controls. The mitotic delay is reproducible and the onset frequency dependent with approximately 14, 90 and 120 days exposure to 45, 60 and 74 Hz electromagnetic radiation required before a significant effect is observed. Removal of affected cultures from the electromagnetic field (75 Hz, 2.0 G, 0.7 V/m) results in the disappearance of the mitotic delay in approximately 40 days. In addition to the mitotic delay, a retardation in reversible protoplasmic streaming was observed at all frequencies.

Radiation Research, in press

REPEATED STRUCTURE OF CHROMATIN IN METAPHASE NUCLEI OF PHYSARUM

Volker M. Vogt and Richard Braun

Institute of General Microbiology, University of Bern
Altenbergrain 21, 3013 Bern, Switzerland

" . . . the size of the DNA in *Physarum* nucleosomes is closely similar to the size of protected fragments obtained from mouse cell nuclei, which we assume to be multiples of 200 nucleotide pairs . . .

" . . . the chromatin in interphase and in metaphase nuclei appears to be equally accessible to micrococcal nuclease.

" . . . we conclude that the basic repetitive structure of chromatin does not change as *Physarum* nuclei pass through mitosis.

FEBS Letters, in press

THE STRUCTURE OF RIBOSOMAL DNA IN *PHYSARUM polycephalum*

Volker M. Vogt and Richard Braun

Institute for General Microbiology, University of Bern
Altenbergrain 21, 3013 Bern, Switzerland

ABSTRACT. The sequences coding for *Physarum* ribosomal RNA are localized on independently replicating, linear DNA molecules of a discrete size, 37×10^6 daltons. Restriction endonucleases $EcoR_I$ and $Hind_{III}$ each cut rDNA into one large and two small fragments. The latter are represented twice per intact molecule, once at each end. Sedimentation and electron microscopic analyses of intact rDNA that has been neutralized from alkaline solution indicate that the entire rDNA molecule has a rotational axis of symmetry near the center. Blocks of short, inverted repetitious sequences appear to be located at the center of the native rDNA and also 3.7 to 11×10^6 daltons flanking the center.

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The Institute of Biology's
Studies in Biology no 56

The Biology of Slime Moulds

J. M. Ashworth
Ph.D., Professor of Biology, University of Essex

and

Jennifer Dee
Ph.D. Senior Lecturer, Genetics Department, University of Leicester

Distributed in the United States by
CRANE, RUSSAK & COMPANY, INC.
347 Madison Avenue
New York, New York 10017

First published 1975
by Edward Arnold (Publishers) Limited
25 Hill Street, London W1X 8LL

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THE MYCETOZOANS

By LINDSAY S. OLIVE
University Distinguished Professor of Botany
Department of Botany
University of North Carolina
Chapel Hill, N.C.

November 1974, 304 pp., $23.50/£11.30
ISBN: 0-12-526250-7

The mycetozoan characteristic of combining within a single life cycle an animal-like feeding stage with a plant-like fruiting stage has fascinated biologists for years. These organisms are easily isolated in the laboratory with minimum of equipment and effort and they are ideal subjects for classroom demonstrations and research projects.

This is perhaps the only comprehensive monograph available on mycetozoans (protostelids, cellular slime molds, myxomycetes) and their associates (plasmodiophorids and labyrinthulids). It consolidates for the first time a mass of scientific information on the classification, life cycles, cell behavior, morphogenesis, ultrastructure, and genetics of these unusual protists.

Techniques for collecting, identifying, isolating, and maintaining mycetozoans are clearly described. Particular emphasis is given to the life cycles of *Dictyostelium discoideum* and *Physarum polycephalum*, organisms that have become favored laboratory subjects for the study of development at the molecular and ultrastructural levels.

Since new taxa continue to be discovered among these organisms, taxonomists and phylogenists will appreciate the practical value of this reference work. THE MYCETOZOANS will also be of great importance to students of mycology, protozoology, developmental morphology, and cell biology. Moreover, the book is ideally suited for use as a textbook, especially for a full course on the mycetozoans and their associates.

Academic Press

(Reviewed by K.B. Raper, ASM News 41, 564, 1975)

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Biochim. Biophys. Acta 402, 363 (1975). (PNL 7, 14, 1975)

E.N Brewer and P. Ting
"DNA Replication in Homogenates of Physarum polycephalum"
J. Cell. Physiol. 86, 459 (1975). (PNL 7, 15, 1975)

J. Dee
"Slime Moulds in Biological Research"
Science Prog., Oxford 62, 523 (1975). (PNL 6, 36, 1974)

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"The Inhibition of DNA Strand Elongation by Cycloheximide in Physarum polycephalum"
Proc. 1975 ICN-UCLA Symp. DNA Synth. Reg., p. 713 (1975). (PNL 7, 15, 1975)

M. Farr
"Some New Myxomycete Records for the Neotropics and Some Taxonomic Problems in the Myxomycetes"
Proc. Iowa Acad. Sci. 81, 37 (1974). (PNL 6, 36, 1974)

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"Correlation Between Tension Force Generation, Fibrillogenesis and Ultrastructure of Cytoplasmic Actomyosin During Isometric and Isotonic Contractions of Protoplasmic Strands"
Cytobiologie 10, 339 (1975). (PNL 7, 16, 1975)

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G. Isenberg, P. Giesbrecht, J. Wecke and K.E. Wohlfarth-Bottermann
"Demonstration of Cytoplasmic Actomyosin Fibrils by the Freeze-Etching Technique"
Microscopica Acta 77, 30 (1975). (PNL 7, 17, 1975)

D.N. Jacobson and W.F. Dove
"The Amoebal Cell of Physarum polycephalum: Colony Formation and Growth"
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"Calcium and Magnesium Contents in Ectoplasm and Endoplasm of Physarum polycephalum Plasmodium"
Protoplasma 86, 169 (1975). (PNL 7, 18, 1975)

R.C. Rustad, N.L. Oleinick and E.N. Brewer
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Exp. Cell Res. 93, 477 (1975). (PNL 7, 19, 1975)

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"Evidence for RNA Linked to Nascent DNA in Eukaryotic Organisms"
Proc. 1975 ICN-UCLA Symp. DNA Synth. Reg., p. 334 (1975). (PNL 7, 20, 1975)

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Protistologica 11, 19 (1975). (PNL 6, 39, 1974)

Additional Articles (continued)

K.E. Wohlfarth-Bottermann
"Tensiometric Demonstration of Endogenous, Oscillating Contractions in Plasmodia of Physarum polycephalum"
Z. Pflanzenphysiol. 76, 14 (1975). (PNL 7, 21, 1975)

THESES

Control of the Differentiated State in Physarum polycephalum

by

Paul Neil Adler

(A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the Massachusetts Institute of Technology May, 1975)

The work described in this thesis had two objectives: to develop further genetic tools for the analysis of cellular mechanisms in Physarum polycephalum, and to begin to study the control of the amoebal and plasmodial states as a model for the control of cellular differentiation.

The life cycle of the acellular slime mold P. polycephalum contains two vegetative forms: small uninucleate amoebae and large multinucleate plasmodia. Plasmodia develop from amoebae by one of two modes: sexual or clonal. In the sexual mode haploid amoebae of different mating type (mt) fuse to form a zygote that develops into a diploid plasmodium by nuclear mitosis. Amoebae carrying the mth allele form haploid plasmodia asexually, a property that is useful for genetic and developmental studies. Heterothallic amoebae do not normally form plasmodia asexually.

The initial part of this thesis reports a method for crossing mth amoebae derived from the Colonia isolate with mt3 or mt4 heterothallic amoebae derived from the Indiana isolate. Strains carrying mt3 or mt4 in a Colonia genetic background have been constructed by a series of backcrosses. The backcrossing resulted in dramatic reductions in heterogeneity of somatic fusion and amoebal plaque size phenotypes, elimination of heterogeneity in plasmodial growth on both defined and rich medium, and a conversion to the Colonia phenotypes.

The progeny of diploid plasmodia formed sexually by crossing two heterothallic amoebal strains were found to include occasional amoebae that formed plasmodia in clones. These amoebae formed plasmodia at a smaller clone size than mth amoebae, but nevertheless could be passaged as amoebae indefinitely. Genetic and cytochemical analysis of these amoebae showed that they were heterozygous for the mating type locus and contained a diploid DNA content. It was also shown that they differentiate into plasmodia without a change in ploidy.

I have also shown that heterothallic amoebae (mt1, mt2, mt3, mt4) form plasmodia (these plasmodia are referred to as illegitimate plasmodia) at a low frequency. These haploid plasmodia are physiologically indistinguishable from plasmodia formed in other ways, if the parental amoebae had been inbred to Colonia. A mating type allele specific pattern was observed with regard to the frequency of illegitimate plasmodium formation at three temperatures. When spores produced by an illegitimate plasmodium are plated in most cases only amoebae of the parental type are obtained. Thus in these cases (which are called phenocopies) the formation of the illegitimate plasmodium does not appear to be the result of a mutation. In a minority of the cases the formation of the illegitimate plasmodium did appear to be the result of a mutation.

Two general classes of mutants have been obtained. In one class the mutant amoebae now form plasmodia clonally at a much higher rate than the parental amoebae (indeed in some cases at a higher rate than mth amoebae). In the other class of mutants the amoebal stage in the life cycle has been eliminated as plasmodia germinate directly from spores.

In summary, I have shown that the amoebal and plasmodial states are compatible with any set of mating type alleles. Thus, the mating type locus controls the probability that an amoeba within an amoebal clone will develop into a plasmodium, but not in an absolute way the differentiated state. The mating type locus must also be involved in the control of sexual plasmodium formation.

Bromodeoxyuridine Incorporation as a Probe for Studying the Nature of Radiation Response of the Slime Mold, Physarum polycephalum

by

Violet A. Breckbill

(A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Department of Biology, Case Western Reserve University, December, 1975)

The effect of the incorporation of 5-bromodeoxyuridine into DNA on the induction of mitotic delay by radiation was studied in the synchronously dividing slime mold, Physarum polycephalum, in its vegetative stage, in an attempt to relate this radiation response to DNA damage. 5-Bromodeoxyuridine was incorporated into the DNA of Physarum polycephalum during (1) the first DNA synthesis period (S-I) following Mitosis I (first mitosis after fusion of microplasmodia) and the extent of unifilar substitution of deoxythymidine by 5-bromodeoxyuridine prior to Mitosis II was determined to be approximately

Breckbill - Bromodeoxyuridine Incorporation as a Probe for Studying the Nature of Radiation Response of the Slime Mold, Physarum polycephalum (continued)

45%; or (2) the DNA synthetic periods following Mitosis I and Mitosis II (S-I and S-II) and the extent of bifilar substitution of deoxythymidine by 5-bromodeoxyuridine prior to Mitosis III was determined to be approximately 75%. Five to 300 μg/ml of the analog was used in the medium for different experiments. Control and 5-bromodeoxyuridine-substituted molds were irradiated with 1000, 2400 and 4300 R (Roentgens) in a [^{60}Co]-γ-irradiator at various times throughout the cell cycle following either unifilar or bifilar incorporation. There were significant increases in radiation induced mitotic delay observed in the molds (unifilar substitution) that had incorporated 5-bromodeoxyuridine from medium containing 100 μg/ml compared to nonsubstituted molds. There were significant increases in the radiation induced mitotic delay at Mitosis IV (with bifilar substitution) in plasmodia that had been grown in medium containing 25 μg/ml of 5-bromodeoxyuridine. When DNA from irradiated molds (harvested during S-II or G_2-II after 40,000 R) was subjected to alkaline sucrose density gradient centrifugation, it was found that the DNA from plasmodia treated with 5-bromodeoxyuridine initially contained more strand breaks than the similarly irradiated control molds. This difference was statistically significant in G_2 and suggests a correlation between the greater initial strand break damage observed in G_2 and the enhancement of radiation induced mitotic delay in the premitotic interval of G_2. No such correlation can be made for events in S with the results of the present study.

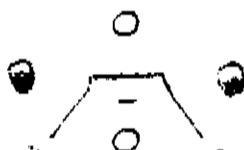
Strong Antigens in Physarum polycephalum

by

Irene Kuhn

(A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science (Molecular Biology) at the University of Wisconsin, 1975)

Five types of antisera were prepared in syngeneic rats, three against amoebal strains and two against genetically related plasmodial strains of Physarum polycephalum. Differences in specificities between the antisera were looked for using immunofluorescence tests and Ouchterlony double diffusion tests. There no detectable differences between any of the three antiamoebal sera, nor were any differences found between the two antiplasmodial sera by either method. However, differences as well as some cross-reactions between anti-amoebal and anti-plasmodial sera were evident.



Gesellschaft für Biologische Chemie

Heidelberg, March 19-22, 1975

E. Dworzak, G. Woertz, W. Linser and W. Sachsenmaier

Effects of Antimetabolites and X-Rays on the Regulation of Deoxynucleoside Phosphorylating Enzymes in Physarum polycephalum

Inhibitors of DNA synthesis stimulate excess production of thymidine (I) and deoxycytidine (II) kinases (EC 2.7.1.75 and 2.7.1.74) beyond the normal activity peak in the early S phase of synchronous multinuclear macroplasmodia of *Physarum polycephalum*.
Similar effects are observed after partial inhibition of RNA synthesis with actinomycin C. The latter effect is most pronounced under conditions which cause mitotic nuclei to be arrested in the metaphase (30 μg actinomycin/ml), preventing them from entering the S period.
X-Rays (1 000 R), which do not interfere with overall DNA synthesis, cause a delayed stimulatory effect on (I). This effect is most pronounced if irradiation occurs during the narrow period between prophase and telophase which also coincides with the most sensitive period for the delaying effect on mitosis. The relative proportions of enzyme variants of (I)[1] are not changed significantly by X-irradiation. Stimulation of (I) by X-irradiation of a mutant resistant against bromodeoxyuridine (BrdUrd)*, which contains less than 10% of wild type (I) activity, is largely reduced corresponding to the low enzyme level. Activity of enzyme (II) is stimulated by X-rays, both in the wild type and in the BrdUrd-resistant mutant, but this effect is not as phase-specific as in the case of (I). Sensitivity to actinomycin of irradiated plasmodia is considerably diminished probably due to repair processes. A model is discussed, suggesting that production of enzymes (I) and (II) is controlled by discontinuous gene activation prior to the onset of mitosis and by a "shut-off" mechanism related to DNA replication in early S phase.

* Courtesy *F. Haugli*, Tromsö Univ.

[1] Gröbner P., Finkenstedt G., Woertz G. & Sachsenmaier, W. (1975) this J. 356, 235.

Address: Prof. Dr. W. Sachsenmaier, Inst. f. Biochemie u. Exp. Krebsforschung d. Univ., Peter-Mayr-Straße 2, A-6020 Innsbruck.

H-S Z. Physiol. Chem. 356, 227 (1975)

P. Gröbner, G. Finkenstedt, G. Woertz, H. Wolf and W. Sachsenmaier

Enzyme Variants of Thymidine Kinase in Physarum polycephalum: Changing Pattern during the Synchronous Mitotic Cycle

Specific enzyme activities of thymidine (I), deoxycytidine (II), and deoxyadenosine (III) kinases (EC 2.7.1.75, 2.7.1.74 and 2.7.1.76) undergo cyclic variations during the synchronous nuclear mitotic cycle in multinuclear plasmodia of *Physarum polycephalum*. Maximum activity of all three enzymes is reached during the early S period comprising a 2- to 4-fold increase over the minimum level in the G_2 period. The bulk of enzyme activity is found in the high-speed supernatant of plasmodial homogenates. Enzymes (I) and (II) are rather unstable in vitro, but may be stabilized with glycerol and bovine serum albumin. Molecular weights were determined by gel filtration and sucrose gradient ultracentrifugation: 70 000 (I); 53 000 (II); 85 000 (III). Chromatography on DEAE-cellulose of plasmodial extracts reveals heterogeneous activity profiles of (I), suggesting the presence of isoenzymes or enzyme variants. This was further confirmed by polyacrylamide gel electrophoresis and isoelectrofocussing. At least three bands (A, B, C) with isoelectric points at pH 7.3, 6.5, and 6.15 may easily be distinguished by this method. The relative amounts of these enzyme variants change drastically during the synchronous nuclear mitotic cycle. Enzyme band (C), with an isoelectric point at pH 6.15, is most pronounced (> 40% of total activity) at the time of mitosis; its relative proportion decreases rapidly during the S period and disappears almost completely (< 5% of total activity) during late G_2 period. It is assumed that during the induction of thymidine kinase, shortly before the onset of mitosis, mainly the enzyme variant (C) is formed, which may be modified subsequently during the cycle giving rise to enzyme variants with higher isoelectric points.

Address: Prof. Dr. W. Sachsenmaier, Institut f. Biochemie u. Exp. Krebsforschung d. Univ., Peter-Mayr-Straße 2, A-6020 Innsbruck.

H-S Z. Physiol. Chem. 356, 235 (1975)

H. W. Sauer, A. Hildebrandt, H. Fouquet, R. Böhme, R. Wick, G. Ernst, K. Scheller, B. Bierweiler and H.-J. Bohnert

Aspects of Coarse Growth Controls in Physarum

Synchronous mitoses and formation of cysts make *Physarum* a suitable system for studies of growth and differentiation.
Chromosomal DNA starts replication in S-phase with predominantly single-copy DNA (euchromatin), while ribosomal cistrons are produced throughout the cell cycle, probably by a rolling circle mechanism.
RNA polymerase A (EC 2.7.7.6) is tightly bound to the nucleolus, binds differentially to nucleolar DNA in vitro, and is significantly (80%) reduced during encystment. Nucleoli from starved cells contain an unchanged number of ribosomal genes and an inhibitor selectively and reversibly affecting enzyme A.
Enzyme B shows little activity on native DNA. Template activity can be increased by an endogeneous elongation factor in vitro.
Nuclear and polysomal RNA contain poly(A)-rich fractions. Cordycepin inhibits synthesis of all classes of RNA and the polyadenylation of cytoplasmic but not of nuclear RNA. Poly(A) stretches from cytoplasmic RNA become shorter with time.
Polysomal, and small molecules of nuclear poly(A) RNA contain fewer redundant base sequences than large nuclear poly(A)-RNA molecules (5 - 10% vs. 20 - 30%).
Poly(A)-RNA from G_2-phase contains more redundant base sequences than that from S-phase (30% or 20%, respectively).
Furthermore, we have evidence for replication-transcription coupling of about half of the poly(A)-RNA fraction in early S-phase.

Address: Prof. Dr. H. W. Sauer, Fachbereich Biologie d. Univ., D-775 Konstanz, Postfach 7733.

H-S Z. Physiol. Chem. 356, 272 (1975)

STUDIES ON THE INHIBITION OF DNA REPLICATION BY CYCLOHEXIMIDE IN *PHYSARUM POLYCEPHALUM*. Helen H. Evans, Sandra Littman, and Thomas E. Evans, Division of Radiation Biology, Case Western Reserve University, Cleveland, Ohio 44106

Inhibitors of protein synthesis have been shown to interfere with DNA replication in many eukaryotic cells, but whether the inhibition is due to a decrease in the rate of chain elongation or to a decrease in the number of replicating units being elongated is a subject of some controversy (for a summary, see Gautschi, J. Mol. Biol. 84, 223, 1974). In this study, treatment of naturally synchronous plasmodia of *P. polycephalum* with cycloheximide was found to cause a decrease in the specific activity of TTP (determined according to Walters, Tobey, and Ratliff, Biochim. Biophys. Acta 319, 336, 1973). During the first 15 min after drug addition, the amount of DNA synthesis -- determined by ^{3}H-thymidine incorporation into DNA corrected for the change in the specific activity of TTP -- was 70% of the control level. Similarly, the molecular weight of progeny strands pulse labeled during the first 15 minutes of the S period in the presence of cycloheximide was approximately 70% of the control (as determined by sedimentation in alkaline sucrose density gradients). Our previous results have indicated that this drug has no effect on either the initiation of replication units or on the ligation of DNA fragments produced by ionizing radiation (Evans, Evans, and Brewer, in Proceedings of the 1975 ICN-UCLA Symposium on DNA synthesis and its Regulation, ed. M. Goulian and P. Hanawalt; W.A. Benjamin, Inc., In Press). It appears, therefore, that cycloheximide inhibits the elongation of progeny strands within replication units, presumably by affecting the synthesis of short-lived proteins necessary for this process. (Supported by NIH Grant GM 19484, U.S.A.E.C. contract W-31-109-ENG-78, and U.S.E.R.D.A. contract AT(11-1)2486.)

Second Annual Colloquium ("Regulatory Biology")
The Ohio State University, Columbus, September 4-6, 1975

STUDIES ON MOTILITY IN PHYSARUM POLYCEPHALUM. D. N. Jacobson, R. M. Johnke, and M. R. Adelman. Duke University Medical Center, Durham, North Carolina 27710.

A wide range of phenomena involving motility can be studied in a single genotype by using the slime mold P. polycephalum. These phenomena include rapid protoplasmic streaming, cell shape changes, translocation of cells along surfaces, and the propulsion of cells by flagella.

Actin and myosin, which may be prepared in high yield and purity, constitute ~5% of the protein of migrating plasmodia and have been identified in shaker-cultured microplasmodia as well as in the haploid gametes of Physarum. While there are important similarities between Physarum and muscle actins and myosins, biochemical studies do reveal significant differences. Plasmodial actin is easily extracted at low ionic strength and actin polymers are stable over a limited range of conditions: thus actin filaments may be metastable in situ. Moreover, since the particle asymmetry of actin aggregates formed in vitro is affected by non-actin "impurities", the actin may exist in alternative polymeric forms in vivo. Plasmodial myosin aggregates less readily than does muscle myosin and has a different

Jacobson, *et al.*, Studies on Motility in P. polycephalum (continued)

polypeptide composition. The myosin ATPase is Ca^{2+}-dependent and has no K^{+}+EDTA activity. The low ionic strength Mg^{2+}-ATPase of plasmodial myosin is strongly activated by plasmodial actin. This activation is Ca^{2+}-independent and various experiments indicate that regulation of the actin-myosin interaction must differ from the various muscle systems now known.

Physarum gametes may exist as either amoeboid or flagellate cells. The culture of gram quantities of amoebae is now possible. Movement of these 7-8μ cells, like PMN locomotion, involves the extension of granule-free cytoplasmic processes and protoplasmic flow. Rapid (15') and synchronous transformation of large populations of the amoebae into elongate (15μ) swimmers can be obtained. This transformation involves several motility patterns including extension of a 3-5μ granule-free process and propagation of waves of protoplasmic constriction. The swimmers retain their actomyosin and contain flagella with the typical "9+2" array of axonemal microtubules as well as an extensive cortical array of cytoplasmic microtubules. These observations are interesting in light of the failure of various attempts to define tubulin in plasmodia or microplasmodia. Supported by NIH grants 5-S04-RR-6148, 2-R01-GM-20141 and NSF grant BMS-74-04967-A01.

Meeting on Cell Motility
Cold Spring Harbor, September 9-14, 1975

Fifteenth Annual Meeting
The American Society for Cell Biology
San Juan, November 11-14, 1975

277. EFFECTS OF LONG-TERM WEAK ELECTROMAGNETIC RADIATION ON PHYSARUM POLYCEPHALUM E.M. Goodman, Michael T. Marron*, and Ben Greenebaum*. Division of Science, University of Wisconsin-Parkside, Kenosha, Wisconsin.

To date, the slime mold Physarum polycephalum has been continuously exposed to a weak electromagnetic field (EMF) of 75 Hz, 2.0 G, 0.7 V/m for more than 1000 days. Cultures exposed to EMF exhibit three significant physiological and biochemical alterations: (1) the time required to complete a mitotic cell cycle increases by one or two hours, (2) the rate of respiration (μl O_2/min/mg protein) is depressed by about 15% and (3) the shuttle streaming period increases. These EMF effects are reproducible and appear after approximately 90 to 120 days of continuous exposure. If EMF affected cultures are returned to a control environment (no EMF exposure) the observed alterations disappear within three to six weeks. Application of the electric (0.7 V/m) component alone resulted in similar but less pronounced effects. The magnetic (2.0 G) component alone had no effect. Completion of either sexual or asexual life cycles is not prevented by exposure to these radiations. (Supported by the Office of Naval Research with funds supplied by the Naval Electronics Systems Command)

J. Cell Biol. 67, 139a (1975)

372. THE EFFECT OF CADMIUM UPON NUCLEAR ACTIVITIES IN PHYSARUM POLYCEPHALUM

Camille Hyde*, M. Frikker*, M. Hertz*, J. Sina* and B. Chin. Department of Environmental and Industrial Health, School of Public Health, The University of Michigan, Ann Arbor, Michigan.

Cadmium is an environmental pollutant of current concern, and has been implicated in mutagenesis, teratogenesis and carcinogenesis. Cadmium toxicity was studied at the cellular level in Physarum polycephalum. At the physiological level, cadmium delays mitosis, with one peak of sensitivity at early-S in the cell cycle and a second peak in mid-G_2. At the biochemical level, exposure to 5×10^{-4} M cadmium ion for 30 min at early-S stimulates the incorporation of tritiated thymidine (H^3-TdR) into DNAse sensitive material by 85% during the period of DNA synthesis which follows. This exposure to cadmium stimulates the rate, the amount and the duration of H^3-TdR incorporation. Conversely, the same exposure at early-S depresses incorporation of tritiated uridine (H^3-UR) into RNA by 45% and of a tritiated amino acid mixture into protein by 17%. Stimulation of TdR incorporation occurs only if exposure to cadmium is made close to or during S, but not if exposure is made in mid-G_2. Effective concentrations of cadmium for stimulation of TdR incorporation lie within 10^{-2} and 10^{-5} M. Stimulation of TdR incorporation is specific for cadmium ion; Mn^{++}, Ni^{++}, Co^{++}, Cu^{++}, Fe^{++}, Pb^{++}, Zn^{++} and Hg^{++} have no effect. These observations, i.e., mitotic delay, stimulation of TdR incorporation, and depression of H^3-UR incorporation into RNA, identify the cell nucleus as a target for cadmium toxicity, and suggest that some of these events may play a role in teratogenesis, mutagenesis and carcinogenesis.

J. Cell Biol. 67, 186a (1975)

376. AMOEBO-FLAGELLATE TRANSFORMATION IN PHYSARUM POLYCEPHALUM.

David N. Jacobson* and Mark R. Adelman. Department of Anatomy, Duke University Medical Center, Durham, N.C. 27710

A method has been developed to synchronize the amoebo-flagellate transformation in large populations of P. polycephalum myxamoebae for phenomenological, ultrastructural, biochemical, and genetic studies of motility, flagellar morphogenesis, and alterations in cellular form. When populations of flagellate swimming cells are plated on an agar surface, the cells assume an amoeboid form and flagella are resorbed within 30 minutes. When these cells are resuspended in buffer, flagella appear on 100% of the cells within 15 minutes and the rounded cells (7-8 μ in diameter) transform into elongate ones (∿ 14 μ in length). Cinemicrographic observations of the transformation of cells adherent to a microscopic slide have revealed several interesting phenomena. The initial event in cell shape change involves extension of a 3-5 μ cellular process which is free of visible cytoplasmic inclusions and which moves across the slide at a rate on the order of 1 μ/sec. Later during the transformation waves of contraction propagate down the elongate cell: about 5 seconds are required for a wave to move one cell length. Initial ultrastructural studies have confirmed that the flagella of the swimmers have a typical "9 + 2" set of axonemal microtubules and have revealed that the elongate cells contain an extensive cortical array of cytoplasmic microtubules. The culture techniques developed for the studies involve the growth of myxamoebae on dense bacterial lawns and allow the routine collection of gram quantities of amoeboid cells. Initial biochemical studies of the amoebae have established that they contain actin and myosin similar to the corresponding plasmodial proteins.
Supported by NIH Grants 5-S04-RR-6148 and 2-R01-GM-20141 and NSF Grant BMS74-04967-A01.

J. Cell Biol. 67, 188a (1975)

534. CA^{++} REGULATION AND INITIATION OF MOTILITY IN MICROPLASMODIA OF PHYSARUM POLYCEPHALUM Larry Matthews* Prgrm. in Biophys. Cytol., Dept. of Biol., University of Pennsylvania, (Introducted by T. Okigaki).

Plasmodia of the slime mold Physarum polycephalum incubated in 10mM caffeine and 10mM Tris maleate buffer, pH 7.1., form spherical microplasmodia (MP) 50-250μm in diameter. These MP are capable of reforming whole plasmodia under the conditions of darkness, high humidity, and 18-22°C. They possess a granular cytoplasm which streams with a variety of morphological patterns within the larger volume of the microplasmodium.

Incubation of the MP in 10mM EGTA Ca^{++}-chelating medium leads to a cessation of streaming and a subsequent spreading of the granular cytoplasm throughout the volume of the MP. Exposure of a MP, "relaxed" by the 10mM EGTA medium, to concentrations of Ca^{++} 10^{-6}M produces a compaction of the diffuse granular cytoplasm into a dense sphere within the larger total volume. This is followed by a resumption of streaming which halts within 30 minutes and the granular cytoplasm again diffuses throughout the entire volume of the MP. Caffeine, which is thought to cause a release of Ca^{++} from the sarcoplasmic reticulum of vertebrate striated muscle, produces identical results in concentrations of 20-50mM. Exposure to 10-100mM NaCl, KCl, or $MgCl_2$ fails to initiate compaction and streaming.

Repeated applications by micropipette of 10mM $CaCl_2$ to the "relaxed" MP in 10mM EGTA produce equally strong responses each time. Repeated applications of 50mM caffeine in the Ca^{++}-free medium elicit progressively weaker responses. Eventually the MP fails to respond. An application of 10mM $CaCl_2$ will now produce a compaction of the diffuse granular cytoplasm and a resumption of streaming. Subsequent applications of 50mM caffeine once again produce an initial strong response followed by progressively weaker responses. These results demonstrate the existence of a Ca^{++}-sequestering system which controls the initiation of streaming in this non-muscle system. (*Supported by grants NIH CA 10 10171-10, NSF BMS 75-00473 A01).

J. Cell Biol. 67, 267a (1975)

540. CHANGES IN ACIDIC NUCLEAR PROTEINS, NUCLEIC ACIDS, AND ULTRASTRUCTURE IN RESPONSE TO HIGH PLASMODIAL DENSITY IN PHYSARUM POLYCEPHALUM L.E. McAlister* V.F. Allison* J.R. Jeter, and C. Nations. Department of Biology, Southern Methodist University, Dallas, Texas, and Department of Anatomy, Tulane Medical School, New Orleans, Louisiana.

Starvation-induced differentiation in the simple eucaryote Physarum polycephalum has been characterized by specific and reproducible changes in the complement of acidic nuclear proteins. When exponentially growing microplasmodia are subjected to conditions of high density, changes in the electrophoretic profile of the acidic nuclear proteins are observed which correspond in part to those induced by starvation. This indicates the possibility of a generalized mechanism for cellular transition from active growth to a non-proliferative cell state. Comparison of the ultrastructural morphology of microplasmodia at high densities with that of starved cultures shows some striking similarities between the two cell states. Nuclei in both cases show an increase in heterochromatic clumping and a decrease in diffuse euchromatic areas relative to nuclei characteristic of exponential growth. Mitochondrial morphology and cytoplasmic organization are also similarly and distinctly altered by starvation and high density. Incorporation of ^{3}H-thymidine into nuclear DNA decreases by 53.2% during the first hour of the high density treatment. DNA synthesis also decreases by more than 50% after eight hours of starvation. No qualitative changes in RNA composition occur in response to high density conditions; however, a quantitative decrease in the 26S fraction is observed.

J. Cell Biol. 67, 270a (1975)

577. CELL CYCLE DEPENDENT POST-TRANSLATIONAL MODIFICATION OF ORNITHINE DECARBOXYLASE. John L.A. Mitchell. Dept. of Biological Sciences, Northern Illinois University, DeKalb, IL 60115

Ornithine decarboxylase is the rate limiting enzyme in the synthesis of the polyamines required for cell growth and proliferation. We have investigated the rapid fluctuations in this enzyme's activity during the synchronous mitotic cycle of Physarum polycephalum to elucidate the mechanism of this precise control of activity. Substrate and coenzyme saturation kinetic studies indicate that this enzyme exists in two forms within the cell, and the relative amount in each form varies with position in the mitotic cycle. Sephadex column chromatography resolved this enzymatic activity into a low molecular weight species (M.W. 90,000) which is activated by low levels of coenzyme (PLP), and a second form of about M.W. 180,000 which is active only at very high levels of substrate and PLP. These forms are readily interconvertible in vitro with high levels of ornithine, PLP and EDTA favoring the transition to the smaller species and the presence of polyamines, putrescine or high salt reverting the enzyme to the high molecular weight form. The data suggest that the active form of this enzyme is the M.W. 90,000 unit which, under adverse conditions of low substrate, high salt, or polyamine accumulation would be inactivated by forming a dimer. Although the dimer form is catalytically inactive at the substrate and coenzyme concentrations of the cell's cytoplasm it can be measured by conversion to the active, monomer form in the high PLP and ornithine concentrations of an appropriate assay buffer. Recent reports indicate that c-AMP is involved in the activation, and thus the dimer to monomer conversion, of this enzyme. Activity fluctuations during the mitotic cycle may therefore result from known variations in these cyclic nucleotides (Supported by grants #74-11 of the Illinois Division of the American Cancer Society and #1-R01-AM 17949-01 from N.I.H.).

J. Cell Biol. 67, 289a (1975)

ADDITIONS TO THE MAILING LIST

Dr. Paul Adler
Center for Pathobiology
Univ. of California at Irvine
Irvine, California 92664 USA

Dr. Roger Anderson
Dept. of Genetics, Univ. of Leicester
Leicester LE1 7RH, ENGLAND

Mr. Donald A. Betterley
Dept. of Botany
University of California
Berkeley, California 94720 USA

Dr. Violet A. Breckbill
Division of Radiation Biology
Department of Radiology
Case Western Reserve University
Cleveland, Ohio 44106 USA

Dr. R.A. Cox
Medical Research Council
Nat'l. Inst. for Medical Research
The Ridgeway, Mill Hill
London NW7 1AA ENGLAND

Dr. John Denbo
Division of Science
Grinnell College
Grinnell, Iowa 50112 USA

Mr. William Dougherty
Dept. of Botany, Univ. of Florida
Gainesville, Florida 32611 USA

Dr. Michael J. Dykstra
Dept. of Botany, Univ. of Florida
Gainesville, Florida 32611 USA

Dr. L. Hall
Inst. of General Microbiology
Altenbergrain 21
CH - 3013 Bern, SWITZERLAND

Dr. Peter Herrlich
Max-Planck-Inst. für Mol. Genetik
Ihnestrasse 63-73
1 Berlin 33, GERMANY

Additions to the Mailing List (continued)

Dr. John C. Hozier
Max Planck Inst. für Virusforschung
74 Tübingen
Spemannstrasse 35 W. GERMANY

Dr. Ulrich Laemmli
Dept. of Biochemical Sciences
Frick Chem. Laboratory
Princeton University
Princeton, New Jersey 08540 USA

Dr. J. G. Lafontaine
Biology Department
Laval University
Quebec 10, P.Q. CANADA

Dr. Ulf Lagerkuist
Dept. of Medical Biochemistry
University of Goteborg
Fack S-400 33 Goteborg 33 SWEDEN

Dr. G. LeMieux
Biochemistry Department
Laval University
Quebec 10, P.Q. CANADA

Dr. L. Rakoczy
Dept. of Plant Physiology
Polish Academy of Sciences
22 Jana St. Cracow POLAND

Dr. Frederick L. Schuster
Department of Biology
Brooklyn College, City Univ. of N.Y.
Brooklyn, New York 11210 USA

Ms. Carol H. Waddell
Dept. of Biochemical Sciences
Frick Chem. Laboratory
Princeton University
Princeton, New Jersey 08540 USA

CHANGES OF ADDRESS

Dr. Kirsten Fink
Department of Biochemistry
University of Leicester
Leicester LE1 7RH, ENGLAND

Dr. Masanao Miwa
Biochemistry Division
Nat'l. Cancer Center Res. Inst.
Tsukiji 5-chome, Chuo-ku
Tokyo, JAPAN